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BIOASSESSMENT METHODOLOGIES FOR THE REGULATORY TESTING OF FRESHWATER DREDGED MATERIAL

Phase II Report

by

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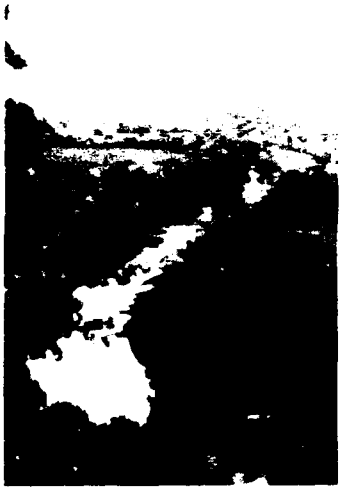

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This report represents the second phase of a 3-year (three-phase) project that the Wisconsin Department of Natural Resources (WDNR) requested from the St. Paul District as planning assistance under Section 22 of the Water Resources Development Act of 1974 (Public Law 93-251). The State of Wisconsin is interested in identifying appropriate bioassessment testing methodologies for the regulatory testing of freshwater sediments scheduled for dredging and open-water disposal.

This Phase II report was generated from a review of the Phase I report by representatives from WDNR and the Corps of Engineers (US Army Engineer Waterways Experiment Station, the St. Paul District, the Detroit District, and the North Central Division). The report includes discussions and recommendations for specific approaches to bioassessment methods in the tiered testing protocol. The methods described in this report have the potential to be the most frequently used testing techniques, and represent the backbone of the tiered testing evaluation for regulatory testing of freshwater sediments.

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PREFACE

This report summarizes a review conducted by the US Army Engineer District, St. Paul, in coordination with the Wisconsin Department of Natural Resources (WDNR), the US Army Engineer District, Detroit, the North Central Division, and the US Army Engineer Waterways Experiment Station (WES). An evaluation was made of the discussions and recommendations in the Phase I document, the technical documents submitted by the attendees, the comments submitted by the participants on various types of testing, and available technical literature relating to bioassessment methodology.

From that review, the St. Paul District compiled this draft report, which includes recommendations for specific approaches for bioassessment methods in the tiered testing scheme. The methods discussed in this draft have the potential to be the most frequently used testing techniques and, as such, represent the backbone of tiered testing evaluation for regulatory assessment of freshwater sediments.

The authors acknowledge the coordinating efforts of Mr. Stan Kummer, project manager for this work effort and Section 22 coordinator for the St. Paul District. Ms. Janean Shirley of the Information Technology Laboratory, WES, edited this report.

This report was written by Dr. Greg Busacker and Dr. Dennis Anderson of the US Army Engineer District, St. Paul, under the general supervision of Dr. Lloyd H. Saunders, Contaminant Mobility and Regulatory Criteria Group, EL, WES. The Chief of ERSD was Mr. Donald L. Robey and Chief of EL was Dr. John Harrison.

Commander and Director of WES was COL Larry B. Fulton, EN. Technical Director was Dr. Robert W. Whalin.

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CONTENTS

BIOASSESSMENT METHODOLOGIES FOR THE REGULATORY TESTING OF FRESHWATER DREDGED MATERIAL

PHASE II REPORT

PART I: INTRODUCTION AND SUMMARY

Introduction

The following report is an analysis of the most promising bioassessment methods for use in the evaluation of freshwater dredged material for open-water disposal proposed during the first year of a 3-year project (Dillon and Gibson 1986). It is a narrow and specific part of an overall initial effort to formulate a plan to manage dredged material from in and around the Great Lakes.

This effort was the second year of a 3-year project that the Wisconsin Department of Natural Resources (WDNR) requested from the US Army Engineer District, St. Paul, as planning assistance under Section 22 of the Water Resources Development Act of 1974 (Public Law 93-251). The State of Wisconsin is interested in identifying appropriate bioassessment testing methodologies for the regulatory testing of freshwater sediments scheduled for dredging and open-water disposal.

The third phase of the project would be to take the recommended methodologies and test them with sediments from the State of Wisconsin. Using this information, regulatory implementation may follow on parts or all of the recommendations. The project has adopted a tiered or hierarchical approach to the evaluation of sediments for disposal options relating to open-water disposal. Criteria for moving from one tier of testing to the next would be decided by appropriate Federal, State, and local authorities.

Summary

The phase I report is a miscellaneous paper (Dillon and Gibson 1986) that summarizes the proceedings of a workshop on April 16-18, 1985, in Milwaukee, WI. The workshop was conducted by the Environmental Laboratory, US Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, for the St. Paul District. The workshop was held in response to a request from WDNR to the St. Paul District for planning assistance under Section 22 of the Water Resources Development Act of 1974 (Public Law 93-251). The State of Wisconsin is interested in identifying appropriate bioassessment testing methodologies for the regulatory testing of freshwater sediments scheduled for dredging and open-water disposal.

A carefully selected set of highly regarded individuals from private industry, the Federal Government, and institutions of higher learning attended the workshop. The participants included both technically oriented individuals who develop and conduct bioassessment tests as well as persons who use the results of such tests in a regulatory decision-making context.

Following the completion of the phase I report, WES assumed more of an advisory role than a planning role, and the technical lead for fiscal year (FY) 1986 was passed to the St. Paul District, with backup support from WES. A meeting was held in Madison, WI, on March 13, 1986,

to discuss the phase I document and to outline the scheduled work for phase II in FY 1986. Attending the March meeting were representatives from WDNR and the Corps of Engineers (WES, the St. Paul District, the Detroit District, and the North Central Division).

At this meeting, the phase I effort was reviewed, and the meeting participants noted that the major conclusions from the experts at the workshop were presented in the phase I document. One major conclusion of the workshop was agreement on the tiered testing approach. The concepts of decision points in the tiered hierarchy and the criteria for defining decision points were not developed. These would be left to local and regional authority negotiations. However, the report identified specific issues associated with bioassessment, tests currently run by experts in the field, and animals used for testing. The work for FY 1986 was outlined as working from and filling out the phase I document, including identification of animals for use in tests, test procedures appropriate for the various tiers, and a presentation of the decision process leading to those choices. The results of this process would be compiled into a phase II report. This phase II report would form the basis for phase III of the Section 22 project, which would comprise the testing and evaluation phase of the recommendations from phases I and II.

The St. Paul District, in coordination with the WDNR, the Detroit District, the North Central Division, and WES, has evaluated the discussions and recommendations in the phase I document, the technical documents submitted by the attendees, the comments submitted by the participants on various types of testing, and available technical literature relating to bioassessment methodology. From this review, the St. Paul District has compiled this draft report, which includes recommendations for specific approaches for bioassessment methods in the tiered testing scheme. The methods discussed in this draft have the potential to be the most frequently used testing techniques and, as such, represent the backbone of the tiered testing evaluation for regulatory assessment of freshwater sediments. This report does not describe in detail additional tests described in the phase I report because these tests are judged to be less useful in a regulatory context. In general, these additional tests are still in the stages of research and development, and there is little general agreement within the scientific community about their specific meaning and utility.

This draft document provides a general discussion of the tiered testing concept, the tiers of the evaluation process, and selected bioassessment tests to be used for those tiers. The detailed protocols for these tests are in the appendixes. Appendix A contains the test protocols for tiers II and III, Appendix B discusses the use of a reference toxicant, Appendix C deals with material handling, and Appendix D describes the ideal daily procedures for laboratory testing. Appendix F discusses animal culture, Appendix G concerns data evaluation, Appendix H outlines health and safety concerns, and Appendix I gives suggested parameters and methods for bulk chemical analysis. In many cases, subjects for the main portion of the phase II report and for the appendixes are identified by heading only. These areas would be addressed in the final report. As of September 30, 1986, this draft represents the current progress towards completion of phase II. It also represents the basis for agreement that the technical direction for phase II is appropriate.

Annotated Summary of Phase I Consensus Agreements

The following 13 items summarize the major agreements reached by workshop participants. The annotations (phase II comments) following each item refer to how the items will be treated from the perspective of phase II.

a. A tiered (hierarchical) testing approach that utilizes tests of increasing complexity and sophistication to reach decisions of greater confidence represents a defensible and technically sound rationale for regulatory decision-making.

   Phase II comment: Adopted.

b. Bulk sediment chemistry analysis gives no indication of bioavailability and therefore no indication of the potential for biological impact. Its primary value lies in providing a qualitative listing of contaminants present in the sediment. One may also use bulk analysis data to calculate the predicted thermodynamically defined maximum bioaccumulation potential for neutral organic chemicals, assuming that specific minimal types of data are gathered during the bulk analysis.

   Phase II comment: Adopted. Minimal types of data include total organic carbon content of the sediment and the concentration of the contaminant in the sediment. The lipid content of the animal that is used for estimation of bioaccumulation potential can be determined or estimated on the basis of previous reports without serious compromise to the value of the estimated bioaccumulation potential.

c. Conducting liquid phase acute lethality tests is generally not necessary unless there is a reason to suspect the water column may be impacted. However, solid phase tests should always be carried out if bioassays are required at all. In laboratory tests, the whole sediment (solid and suspended phases) should be considered in experimental design and data interpretation.

   Phase II comment: Use of whole sediment for bioassessment testing is recommended.

d. A matrix of organisms, as opposed to single species testing, should be used. It should include animals living in sediment (amphipods, larval chironomids, and mayflies, etc.) as well as those associated with the benthic substrate (mysids, daphnids, fish, etc.). It is desirable but not required technically that species selected for acute lethality testing should also be able to be used in sublethal and bioaccumulation assessments.

   Phase II comment: Recommended animals for acute and life cycle testing are the daphnid *Daphnia magna*, the midge *Chironomus tentans*, and the amphipod *Hyalella azteca*. These animals are adaptable for use in more than one tier of testing. However, because of the quantity of tissue needed for analysis, depending on the contaminants of concern, alternative organisms may be required for the bioaccumulation testing.

e. Any regulatory testing program would benefit by the routine use of standard reference toxicant bioassays to assess the sensitivity of test organisms.

   Phase II comment: Use of standard reference toxicant is recommended (Appendix B).

f. The decision to use site water and flowing water exposures in biological tests should be made on a case-by-case basis, but the first consideration must always be meeting the needs of the test organism.

   Phase II comment: The needs of the recommended test organisms are met in the proposed test procedures.

g. Bioaccumulation of contaminants may be evaluated at three different tiers: (1) mathematical calculation of a thermodynamically defined maximum, (2) short-term laboratory

tests to indicate the potential for uptake and the prediction of steady-state levels, and (3) long-term laboratory tests to empirically determine steady-state concentrations and the factors affecting bioaccumulation.

Phase II comment: The proposed protocols allow for the determination of partitioning between sediment and water, sediment and animal, and water and animal. Bioaccumulation may be determined by calculation or empirically.

h. The decision to purge the guts of organisms used to evaluate bioaccumulation potential following their removal from sediments must be made on a case-by-case basis. Gut purging should not be used in trophic transfer studies.

Phase II comment: Adopted.

i. Bioaccumulation, bioconcentration, biomagnification, and trophic transfer are complex interactive processes that are difficult to test in the laboratory and impossible to accurately separate and identify in the field. Laboratory trophic transfer studies, which are simple and therefore easy to decipher, may be desirable if there is an important predator-prey relationship existing in or near a disposal area.

Phase II comment: Adopted. Test design for trophic transfer would be done on a case-by-case basis.

j. Life cycle tests in which growth and reproduction are determined should receive the highest priority of all nonlethal bioassessment methodologies.

Phase II comment: Adopted. The relationship of the "intrinsic rate of population growth (r)" to life cycle tests would be considered useful for tier IV under other bioassessment techniques. *It may prove useful for routine culture monitoring* if testing laboratories keep track of adult mortalities and the production of neonates.

k. Some sort of oncological assessment is highly desirable in light of the public's concern for this issue. Although there are some potentially useful assessment methods that are in various stages of development and refinement (e.g., modified Ames test, aryl hydrocarbon hydroxylase induction, and tumor induction in medaka killifish), there is no generally accepted test currently available for routine regulatory testing.

Phase II comment: The State of Wisconsin is currently evaluating the usefulness of the Ames test for routine sediment screening. Its use is recommended in this report. Interpretation of results would be a decision for the local authority.

l. A number of other bioassessment methods may be considered on a case-by-case basis. Although they probably would not be used on a routine basis, they could be used when additional biological evaluations are warranted.

Phase II comment: These methods are discussed under Part III, RECOMMENDED PROCEDURES FOR TIERS, TIER IV.

m. A consensus tiered testing program that incorporates physical and chemical information and acute and chronic bioassessment tests as well as methods to determine bioaccumulation potential was developed by the workshop participants. Lacking in this hypothetical testing program are the quantitative keys that dictate at what point one moves from one tier to the next. The formulation of these decision criteria would be driven by the more

general decision made by local authorities of what level of environmental protection is desired (e.g., some degradation is acceptable, no further degradation, or a return to pristine conditions).

Phase II comment: It would be recommended that the decision criteria be developed based on economic considerations, engineering feasibilities, and applicable laws and regulations.

PART II: TIERED TESTING

Background and Discussion from Phase I

During the phase I workshop, several participants mentioned that they submitted pre-workshop input about a tiered (hierarchical) testing approach for sediments. This approach was also mentioned in other inputs, and, as a consequence, it was adopted as a framework for the phase I workshop. This tiered testing approach, which uses tests of increasing complexity and sophistication to reach decisions of greater confidence, represents a defensible and technically sound rationale for regulatory decision-making.

The phase I workshop presented the tiered testing format as originating from the testing protocol for hazard assessment (HA) proposed by Cairns, Dickson, and Maki (1978). Two major blocks of information regarding the effects assessment and the exposure assessment provide the framework for HA. A series of tiers provides tests that advance from the simple to the more sophisticated. As one advances from one tier to the next, an increase in time and effort is generally required. However, more confidence is gained in the decision made from such data. The workshop participants felt that this type of approach would permit a more efficient use of resources and result in a concomitant reduction in unnecessary testing. Thus, the workshop reached an agreement that the tiered approach offers a sound scientific rationale for making decisions, and that concept is endorsed in the draft report.

Structure of Tiered Testing Approach

Tier I

The recommended structure for the tiered testing approach is shown on page 10. Briefly, tier I includes initial activities that are common to the evaluation phase of any proposed dredging project. There would be a search for historical records, examination of the site, inputs from other agencies, and evaluation of existing data. The emphasis would be on determining if there was a reason to believe that disposal of dredged material would result in an unacceptable adverse effect on the environment. The evaluation process could stop at this point. Alternatively, additional testing may be warranted.

Tier II

Under tier II, screening methodologies designed for a rapid evaluation would be employed. The first method to be applied probably would be a chemical inventory of selected parameters. However, this inventory would indicate presence in the sediment only and could not suggest whether contaminants present would be available to benthic organisms. A second step of this tier would seek to evaluate the bioaccumulation potential of environmental contaminants of concern (for example, a predictive calculation of the maximum possible tissue concentration of neutral organic contaminants such as polychlorinated biphenyls (PCBs) using the organic carbon content of the sediments and the lipid content of the test organisms as normalizing factors, because in general, neutral organic contaminants that would tend to accumulate in animal lipids (e.g., hydrophobic compounds) are less available for uptake from sediments with high organic carbon content). A third step would employ an acute lethality examination of the potential for the

sediment to rapidly kill sensitive benthic and epibenthic invertebrates such as midge larvae and cladocerans. The final test of this tier is the Ames test, which uses bacteria to assess the mutagenic potential of sediment contaminants (Allen, Noll, and Nelson 1983).

The results from this tier of tests might preclude further testing. For example, if bulk chemical analysis showed a presence of contaminants and if the sediment proved to be acutely lethal to test organisms, the local and regional authorites could decide to end testing at this point. Likewise, a finding of "no contaminants" could also end the evaluation. If the sediment is not acutely lethal but some potential for bioaccumulation exists, then one might choose to test for effects on growth and reproduction in tier III. On the other hand, calculated maximum tissue concentrations may be below acceptable levels indicating the dredged material is acceptable for open-water disposal. What constitutes acceptable or nonacceptable material is determined by Federal, State, and local regulatory authorities.

Tier III

Tier III involves an examination of the chronic effects on growth and reproduction as well as a laboratory determination of bioaccumulation potential. The tests are more expensive to conduct but provide more comprehensive and detailed information, and therefore a more technically sound decision. For instance, one can determine long-term effects on mortality rates, potential effects on growth that relate to the general physiological well-being of the test organisms, and the population's potential to remain self-sustaining. Survivors of the chronic exposures could be analyzed for selected chemicals chosen in the bulk chemistry analysis. In this phase, it might be found that a given contaminant with a high degree of bioaccumulation potential is so tightly bound in the sediments that this contaminant is unavailable to inhabiting organisms. The evidence would be a lack of accumulation in the survivors of the chronic exposure.

This tier does not establish a link between specific contaminants and biological effects. When working with complex mixtures such as sediments, it is technically indefensible to draw causal relationships between tissue residues of environmental contaminants and observed biological effects.

Tier IV

Tier IV testing would be rare and not a routine event in regulatory evaluation of sediments. In general, regulatory decisions for most dredging projects would be made long before testing reached tier IV. Tier IV would generally be appropriate only for the more controversial and/or larger projects. This level of testing will use more sophisticated tests that are generally not appropriate for routine testing. This approach would involve a much higher testing cost. In tier IV, other bioassessment techniques such as effects on bioenergetics, histopathology, or microcosms might be examined. In general, these tests are more in the line of state-of-the-art investigations. The phase I workshop participants felt these tests would be most useful when viewed in conjunction with test results from the earlier tiers to make a more informed and technically defensible decision. Laboratory determinations of steady-state concentrations of contaminants in sediments and exposed animals could be determined by using the same exposure methodology described for chronic exposures and sampling over extended periods of time rather than just at the end of the test.

PART III: RECOMMENDED PROCEDURES FOR TIERS

Recommended Structure

The following represents the recommended structure for phase II.

Tier	Activity*
I	Initial assessment: historical inputs, siting, identification of existing data, etc.
II	Bulk chemistry Predictive calculation of bioaccumulation potential (rapid) Acute lethality** Ames test (rapid)
III	Chronic life cycle test (growth and reproduction)** Laboratory determination of bioaccumulation potential**
IV	Laboratory determination of steady-state concentrations and important factors affecting bioaccumulation** Trophic transfer potential Other bioassessment techniques: bioenergetics, aryl hydrocarbon hydroxylase (AHH) induction bioassay, sister chromatid exchange (SCE), adenylate energy charge (AEC), microcosms

* No significance is attached to order of presentation within tiers.
** These tests could conceivably be combined into a single text.

Tier I

Tier I consists of a historical investigation for existing information regarding a site scheduled for dredging. This information would be used to make the initial assessment regarding the need for testing. Information could be gathered from the following list of sources and others as appropriate: (a) agency records such as State and Federal Environmental Protection Agencies, State Departments of Natural Resources; (b) surveys of local land use (industrial, agricultural, mining, urban); (c) surveys of potential point and nonpoint pollution sources, nearby and in contiguous watersheds; (d) historical land-use patterns; and (e) previous surveys, either at the site or nearby, that were conducted by State and Federal agencies or any other source.

Compiled information would be used to make a judgment regarding the need for additional information. If there was no reason to believe that disposal of dredged material would result in unacceptable adverse environmental effects, the evaluations could stop here. If there was reason to believe that an adverse effect might occur, further evaluation under tier II would be appropriate.

Bulk chemical analysis

Decisions on the selection of disposal alternatives for dredged sediments have historically depended on bulk chemical analysis to a large degree. Bulk chemical analysis provides estimates on the total concentrations of constituents in the sediment sample, but these estimates are poorly related to the biological availability of the constituents (Plumb 1981). Also, bioavailability may vary among different sediments and different contaminants (Dillon and Gibson 1986), and unknown toxicants may be present that were not included in the analysis. As long as one recognizes the shortcomings of this type of analysis, bulk chemical data may be useful for the initial assessment phase since it can indicate the presence or absence of specific contaminants. Also, these data can be used for generating the predictive calculation of the maximum bioaccumulation potential (Dillon and Gibson 1986). The consensus reached by the phase I participants was that, historically, major emphasis has been placed on the bulk chemistry data and that these data have been interpreted in a technically unsound fashion (Dillon and Gibson 1986). The technique does have usefulness during initial evaluations, particularly for determinations of organic carbon, grain size, and moisture content that are needed for predictive calculations of bioaccumulation potential. Bulk chemical analysis can be followed by bioassessment methods to determine overall toxicity of sediments and to look at bioaccumulation of specific chemicals.

Overall, it is recommended that bulk chemical analysis be used as an initial evaluation tool. If sediments do not appear to contain toxic concentrations of contaminants, the evaluation may end at this point. In sediments that may be marginally toxic, bioassessments should be used to assess toxicity. If sediments appear to be highly contaminated, the evaluation process may end and bioassessments would not be run; alternate disposal methods would be used.

Methodology for conducting bulk chemical analysis and a suggested list of parameters for consideration are included in Appendix I.

Predictive calculation of bioaccumulation potential

A straightforward calculation for predicting the bioaccumulation potential of hazardous chemicals is desirable for regulatory applications. Many organic compounds have a greater solubility in animal lipids than in water and are more likely to accumulate in organisms in concentrations that exceed those found in the environment. Bioaccumulation of contaminants can occur by three main pathways: (a) direct absorption from water by respiratory tissues; (b) absorption directly from water or sediment across the integument; and (c) through ingestion and subsequent absorption across the lumen of the gut (Rubinstein, Gilliam, and Gregory 1984).

There are procedures and calculations for predicting animal tissue uptake from a strictlly aqueous medium using the octanol/water partition coefficient (McFarland 1984; McFarland and Clarke 1986) or using drug transport methods (Spacie and Hamelink 1982). When examining transfer of contaminants from sediment to animal tissues, it has been found that some compounds are not available for transfer because of adsorption or complex formation with sediment organic carbon (McFarland 1984) and/or particulate material (McFarland and Clarke 1986, 1987).

From these investigations, a thermodynamic approach has been proposed for predicting the thermodynamically defined maximum bioaccumulation potential (TBP) of neutral organic compounds from sediments (McFarland 1984; McFarland and Clarke 1987; Appendix A). Verification of this approach is in progress and the results seem promising (McFarland 1984).

This approach views bioaccumulation as a redistribution of contaminants between oganisms (McFarland 1984). The following assumptions are made: (a) no kinetic or steric hindrances prevent the establishment of a steady state between the sediment and the organism; (b) the concentration accumulated by organisms is lipid-dependent; and (c) all of a given contaminant in sediment is available to organisms when sediments are normalized for organic carbon.

The use of TBP in tier II would allow a first-step evaluation of the bioaccumulation potential of sediments. If the calculated TBP is less than a concentration that would cause concern, then further testing might be omitted. If the calculated TBP is of concern, then further testing under tiers II and III would likely be completed before an investigation of steady-state concentrations in the laboratory would be done under tier IV; the latter would be used to predict final steady-state tissue levels (McFarland 1984).

Acute lethality test for tier II (*Daphnia/Chironomus*)

Introduction. Acute toxicity of sediments to aquatic macroinvertebrates would be assessed by a 48-hr exposure of two invertebrate species to sediment samples. One species, *Daphnia magna*, represents organisms inhabiting the near-bottom and water-column environment; the other species, *Chironomus tentans*, represents the organisms inhabiting the in-sediment environment.

The exposure would be conducted with whole sediment in the exposure vessel. Initial preparation of the sediment (shaking for 24 hr) would subject *D. magna* to near-maximum concentrations of contaminants similar to concentrations that might be found in the interstitial water of the sediment. *C. tentans* burrows in sediment and would be exposed to interstitial water and to contaminants sorbed to sediment particles. Lethality would be the monitored end point. Sublethal effects on behavior and activity patterns would be noted but would not be considered the primary toxic effects.

Test summary (Ziegenfuss and Adams 1985). The procedure would determine acute toxic effects of chemicals sorbed to sediments using *Daphnia magna* and *Chironomus tentans* in a 48-hr period. Each treatment would be replicated four times using five *D. magna* and five *C. tentans* per replicate, for a total of 20 *D. magna* and 20 *C. tentans* per treatment. *D. magna* (<24-hr old) and *C. tentans* (second instar) larvae would be exposed in the same bottle to a reference sediment, a control sediment, and five concentrations (proportionally diluted with reference sediment) of the sediment to be tested under static conditions. A 50-g sample of sediment and 200 ml of water would be placed in a wide-mouth polycarbonate centrifuge bottle, shaken for 24 hr, and centrifuged for 15 min at 2,000 rpm (500 × G) to clarify the water column yet not pack the sediment to the point where invertebrates could not burrow. Mortality checks would be made at 24 and 48 hr for *D. magna* but only at 48 hr for *C. tentans*. The EC50 (median effective concentration) and the NOEC (no observed effect concentration) would be determined (American Public Health Association (APHA) 1985; US Environmental Protection Agency (USEPA/US Army Corps of Engineers (USACE) 1977; Litchfield and Wilcoxon 1949).

General advantages. The use of *Daphnia* sp. as bioassay organisms is widespread in the United States, Canada, and Europe. Daphnids are sensitive to a wide variety of toxicants and have been used to set water quality criteria (Buikema, Geiger, and Lee 1980; National Academy of Science 1972) and to bioassay test sediments (Ziegenfuss and Adams 1985; Adams et al. 1985; Nebeker et al 1984). Culture methods are simple and well established (Lawrence 1981; Goulden et al. 1982; US Environmental Protection Agency 1986a).

Chironomus tentans also have been widely used for water quality and sediment testing (Ziegenfuss and Adams 1985; Adams et al. 1985; Nebeker et al. 1984; Adams, Kimerle, and Mosher 1985; Anderson 1980) and the culture methods of this midge are well established (Lawrence 1981; Nebeker et al 1984; Batac-Catalan and White 1982).

Specific advantages. Specific advantages of the acute lethality test are as follows:

a. The test uses the multispecies approach recommended by phase I.

b. The test would expose the organisms to a potentially maximum exposure condition for chemicals likely to enter the water column from the sediment.

c. The test would also expose benthic organisms to the possibility of direct absorption of contaminants across the integument.

d. The test is logistically simple to execute and offers the opportunity to examine partitioning of chemicals between the interstitial water and the sediment.

Disadvantages. Differences in laboratory water supplies may make inter-laboratory interpretations difficult (Maciorowski and Clarke 1980).

Ames test for tier II

Introduction. Use of the Ames test for determining the mutagenic potential of sediments has been advanced by Allen, Noll, and Nelson (1983) and was discussed at length by the phase I participants (Dillon and Gibson 1986). In general, the consensus was that the Ames test had potential application as a screening tool. However, concerns were expressed about the utility of the assay and its interpretations: (a) meanings of end points would be unclear; and (b) there would be a need for an unbiased interpretation of end points along with socioeconomic input.

Mutagenic compounds induce mutagenic events in a number of ways that are not detected by the Ames test. Generally, mutagenic events may cause the following results: (a) the production of proteins that may not function or that may function at a reduced rate because of incorrect amino acid sequences; (b) blocked production of proteins because of interference in critical biochemical pathways; (c) incorrect deoxyribonucleic acid (DNA) replication during mitosis and subsequent dysfunction (death) of daughter cells; and (d) incorrect DNA replication during meiosis and subsequent dysfunction of gametes or the product of sexual reproduction.

The specific mechanisms of mutagenesis (Rossman 1981) include the following: (a) misreplication caused by altered DNA due to the interaction of the mutagen and DNA; (b) misreplication of DNA caused by decreased fidelity of DNA polymerase; (c) inhibition of proofreading caused by inhibition of a 3'-5' exonuclease function that excises mismatched nucleotides (the mutagen could interact at several places in this case); (d) the induction of an error-prone DNA repair system (SOS system) caused by mutagen-induced lesions on DNA that prevent base-pairing of any kind at the damaged site; and (e) joining of mutagens with other compounds to become comutagens because of a mutagenic effect that would not be seen with the

compounds above, or in some cases comutagens interfere with DNA repair processes where DNA repair is possible.

The unique approach of separating sediment extracts on thin layer chromatography plates in conjunction with the Ames test has proven to be semi-quantitative for use in sediment organic extractable organic material (Reilly, O'Connor, and Boone 1986). However, it does work very well for airborne particulate organic matter (Butter, Kneip, and Daisey, in preparation).

Techniques that may prove useful in the future include prophage induction in bacteria (Rossman, Molina, and Meyer 1984) and the use of a rather ubiquitous free-living nematode with a 96-hr postembryonic development period (Samoiloff et al. 1980).

Advantages. Advantages of the Ames test are as follows:

a. The Ames test has few false positives compared to other genotoxicity assays.

b. The Ames test is relatively inexpensive to run.

c. Aside from metals, the Ames test detects greater than 90 percent of mutagens tested.

Disadvantages. Disadvantages of the Ames test are as follows:

a. The Ames test does not detect all mutagens (e.g., metals, polychlorinated pesticides).

b. The utility of the Ames test for sediments is unproven.

Tier III

Introduction

Chronic toxicity of sediments to aquatic macroinvertebrates would be assessed by three exposure protocols using three species of aquatic invertebrates. One species, *Daphnia magna*, represents organisms inhabiting the near-bottom and water-column environments. The second species, *Chironomus tentans*, and the third species, *Hyalella azteca*, represent the organisms inhabiting the in-sediment environment. The major difference between the second and third species is in the reproductive phase of the life cycle. *H. azteca* spends its entire life cycle in water and sediment while the adult phase of *C. tentans* is terrestrial.

In all cases, exposure would be conducted with whole sediment in the exposure vessel. Monitored end points would be survival, reproduction, and growth. Static exposures with supplemental aeration would be used for all three species. The test with the midge *C. tentans* may also involve a flow-through exposure providing a contrast with effects under static conditions.

Chronic life cycle test for tier III (*Daphnia*)

Test summary (Nebeker et al. 1984). The procedure would determine chronic toxic effects of chemicals sorbed to sediments using *Daphnia magna* in a 10-day period (Nebeker et al 1984). Each treatment would be replicated twice using 20 *D. magna* per replicate, for a total of 40 animals per treatment. A chronic toxicity test with this organism would not be started until a 48-hr EC50 had been determined with the animals fed during the test.

D. magna (5 days old) would be exposed in glass jars with 2.5 L of water plus a 500-ml volume of suspected toxic test sediment. The exposure would consist of a reference sediment, a

control sediment, and five concentrations of the sediment to be tested (proportionally diluted (w/w) with reference sediment) under static conditions. At the end of 10 days, surviving adults and young would be counted.

General advantages. The use of *Daphnia* sp. as bioassay organisms is widespread in the United States, Canada, and Europe. Daphnids are sensitive to a wide variety of toxicants and have been used to set water quality criteria (Buikema, Geiger, and Lee 1980; National Academy of Sciences 1972) and to bioassay test sediments (Ziegenfuss and Adams 1985; Adams et al. 1985; Nebeker et al. 1984). Culture methods are simple and well established (Lawrence 1981; Goulden et al. 1982).

Specific advantages. Specific advantages of the chronic life cycle test include:

a. The test period is short (10 days).

b. The test exposes daphnids from subadult to maturation.

c. *Daphnia* would release three broods during the 10-day period.

d. There would not be secondary reproduction by the first release brood.

e. The test would expose the organisms to a potentially maximum exposure condition for chemicals likely to enter the water column from the sediment.

f. The test is logistically simple to execute and offers the opportunity to examine partitioning of chemicals between the interstitial water and the sediment, by separating the remaining water and sediment using centrifugation, then analyzing for the selected chemical.

Disadvantages. Young daphnids may offer significant competition to adults if sufficient food is not provided.

Chronic life cycle tests for tier III (*Chironomus*)

Test summary (Mosher, Kimerle, and Adams 1982). The procedure would determine chronic toxic effects of chemicals sorbed to sediments using *Chironomus tentans* in a 14-day period. A chronic toxicity test with this organism would not be started until a 48-hr EC50 had been determined with the animals fed during the test. *C. tentans* would be exposed over 14 days of their life cycle (second to fourth instars) in glass test chambers with 2 L of water plus 100 g of the suspected test sediment under static and flow-through conditions. The exposure would consist of a reference sediment, a control sediment, and five concentrations of the sediment to be tested (proportionally diluted (w/w) with reference sediment). Larvae would be fed daily. At the end of 14 days, survivors would be counted and individually weighed. The EC50 and the NOEC would be determined for surviving larvae (APHA 1985; USEPA/CE 1977; Litchfield and Wilcoxon 1949). Effects on growth and survival would be determined for each treatment. Partitioning coefficients could be determined for each test in the static exposure by using selected chemical analysis following centrifugal separation of the remaining water and sediment, as well as analyzing the survivors for selected chemicals.

General advantages. *Chironomus tentans* have been widely used for water quality and sediment testing (Ziegenfuss and Adams 1985; Adams et al. 1985; Nebeker et al. 1984; Adams, Kimerle, and Mosher 1985; Anderson 1980), and their culture methods are well established (Lawrence 1981; Nebeker et al. 1984; Batac-Catalan and White 1982).

Specific advantages. Specific advantages of the chronic life cycle tests for tier III (*Chironomus*) are as follows:

a. The test would contrast a worst-case static exposure with a flow-through exposure.

b. The test would allow comparisons of partitioning between animal and sediments under the two conditions.

c. The test would expose benthic organisms to the possibility of direct absorption of contaminants across the integument.

d. The test is logistically simple and allows for the determination of bioaccumulation.

e. The time needed for the test is only 14 days.

Disadvantages. The disadvantages of chronic life cycle tests for tier III (*Chironomus*) are as follows:

a. Expected mortality in controls may reach 15 percent.

b. The assays involve standard laboratory protocols and may not represent field conditions.

c. Differences in laboratory water supplies may make interlaboratory interpretations difficult (Maciorowski and Clarke 1980).

Chronic life cycle test for tier III (*Hyalella*)

Test summary (Nebeker et al. 1984). The procedure would determine chronic toxic effects of chemicals sorbed to sediments using *Hyalella azteca* in a 28-day period (Nebeker et al. 1984). Each treatment would be replicated twice using 50 *H. azteca* per replicate, for a total of 100 animals per treatment. A chronic toxicity test with this organism would not be started until a 48-hr EC50 had been determined with the animals fed during the test.

Adult *H. azteca* would be exposed in 20-L glass aquaria with 2 to 3 cm of sediment on the bottom with an overlay of 15 cm of water. The exposure would consist of a reference sediment, a control sediment, and five concentrations of the sediment to be tested (proportionally diluted (w/w) with reference sediment) under static conditions. Animals would be fed twice a week during the 28-day period. At the end of 28 days, surviving adults and young would be screened from the sediment and counted.

The median effective concentration (EC50), the no-observed-effect concentration (NOEC), the number of young produced, and survival percentage would be noted (APHA 1985; USEPA/CE 1977; Litchfield and Wilcoxon 1949). Effects on reproduction and survival would be determined for each treatment. Partitioning coefficients could be determined for each test in the static exposure.

Advantages and disadvantages. *Hyalella azteca* is preferred because of its continuous reproduction, rapid growth, and behavior of burrowing in sediment when disturbed. Also, they are not as prone to cannibalism as are some other amphipods.

H. azteca is nearly ubiquitous throughout North America in permanant bodies of water (Lawrence 1981). Although there have been problems with consistency and culture when *H. azteca* has been used in toxicological experiments, these problems have been addressed and

overcome (Lawrence 1981; Nebeker et al. 1984). Under culture, *Hyalella* will periodically require thinning because of rapid expansion (Nebeker 1984).

Pontoporeia hoyi, a benthic freshwater amphipod, was highly recommended by the phase I workshop participants. It was dropped from further consideration, however, because of its narrow temperature preference, and requirements for almost continual darkness. The animals require a temperature of 4° C (Landrum*; Landrum et al. 1985); they will tolerate cooling of several degrees during transport but will not tolerate warming.* They can be acclimated gradually over the temperature range of 4°-15° C (Landrum 1982), but their condition becomes marginal when held at 7° C and above.

Specific advantages. Specific advantages of chronic life cycle tests for tier III (*Hyalella*) are as follows:

a. The test can be run under static conditions approximating worst-case exposure.

b. The animals spend their entire life cycle associated with sediments.

c. Culture procedures allow for the production of large numbers of animals of the same age and size.

d. There will be no secondary reproduction by released young.

e. The length of the test will allow for growth of the released young, facilitating estimates of growth and survival.

f. The test allows for determination of bioaccumulation.

Disadvantages. Specific disadvantages of chronic life cycle tests for tier III (*Hyalella*) are as follows:

a. The test is long (28 days).

b. The small size of the young will make them difficult to count.

Rejection of other species of amphipods. Other amphipod species would not be acceptable for testing for the following reasons:

a. Growth in the laboratory is highly variable.

b. Cannibalism is a problem.

Laboratory determination of bioaccumulation potential

The laboratory determination of bioaccumulation potential can be done in two ways: The first way involves the exposure of organisms to sediment using the chronic procedures already given in this section. This method demonstrates that the chemicals in the sediment are in a bioavailable form, but is not a quantitative measure of bioaccumulation. In the second method, aquatic organisms would be exposed to contaminated sediment in the laboratory for a period of time that is sufficient to allow bioaccumulation to occur. To achieve steady state, in which the

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* Personal Communication, P. F. Landrum, National Oceanic and Atmospheric Administration, Great Lakes Environmental Research Laboratory, Ann Arbor, MI.

maximum bioaccumulation level occurs, the test may need to be conducted for a minimum of 28 days depending on the chemical(s) under consideration. An alternative to running the test this long is to make an estimate of steady state concentration in tissues by using a kinetic model (McFarland and Clarke 1987). "Uptake kinetics" can be examined by periodically terminating exposures during the 28 days and measuring concentrations in survivors. For instance, animals might be collected for analysis at 0, 1, 2, 3, 5, 9, 15, 22, and 28 days. The information from this constant sequential exposure will enable the data to be fitted to a model so that steady state can be projected, relative to the exposure. This will result in a measurement of bioaccumulation potential.

Tier IV

Introduction

Tier IV involves the use of bioassessment techniques that are not commonly used because of complexities of approach and analysis, and the additional expense incurred for long-term investigations. However, some dredging projects which are large and/or controversial may require additional technical information to increase the confidence associated with the ultimate management decision. This is a basic tenet of the tiered testing approach. A number of techniques having applicability to bioassessment of sediments for open-water disposal will be given. Their usefulness for evaluation of projects would be made on a case-by-case basis, largely depending on circumstances and the class of contaminants involved. In general, a further search for methods will need to be done; and, before testing is started, agreement between the user and regulatory authorities should be made concerning procedures and analysis. No methods for tier IV will be presented in the appendixes of this volume.

Steady-state concentrations

Laboratory determinations of steady-state concentrations and important factors affecting bioaccumulation would involve exposures of invertebrates and fish to contaminated sediments. An estimate of steady-state concentrations can be done by the use of a kinetic model, or an actual measurement of steady state can be made by monitoring tissue levels.

Trophic transfer potential

Determinations of trophic transfer potential would involve feeding organisms carrying a body burden of a contaminant to an organism occupying a higher trophic level. Purging of the digestive tract of prey organisms is recommended prior to their ingestion by a predator. Trophic transfer refers to increases in tissue residues through successive trophic levels. The subject of trophic transfer is extremely complex, and simple experimental designs such as sediment-invertebrate-fish are recommended. Information gathered could be used to evaluate and expand bioaccumulation results. It may also be more useful for understanding cause and effect observed in other tiers rather than for use in prediction (Dillon and Gibson 1986).

Intrinsic rate of population growth (r)

Measurements of the rate of population increase in the face of sediment contaminants may be particularly useful in the evaluation of toxic effects to benthic invertebrates (Dillon and Gibson

1986). The intrinsic rate of population growth (r) is based on observations of survival and fecundity (Caughley 1977; Daniels and Allan 1981). This rate is best expressed as a logistic model with r being the exponential rate of population increase in a population with a stable age distribution (Caughley 1977). This model is attractive because it moves away from examination of individual responses to the examination of population responses, which may be more sensitive to long-term trends in population survival. The uses of r, population life tables, and fecundity rates have been examined and found to be promising (Dillon and Gibson 1986; Daniels and Allan 1981; Gentile 1985). The tests proposed in tier III of this volume do not allow sufficient time to determine these patterns.

Other bioassessment techniques

Bioenergetics. Bioenergetic measurements are generally designed to answer mechanistic questions regarding altered growth or potential for growth. The use of bioenergetics to investigate toxic effects of sediments has been described as more of a research item: (a) it may be used for evaluation of other tests; (b) it may have limited utility for expanding bioaccumulation; and (c) its major use may be for understanding cause and effect rather than prediction (Dillon and Gibson 1986). Bioenergetic measurements have been used with some success to investigate the impact of dredged material on benthic species (Johns, Gutjahr-Gobell, and Schauer 1985).

Histopathology. Histopathology has long been advocated as an effective tool for examining histopathological effects in organisms exposed to sediments, and it has recently been reevaluated (Yevich et al. 1986). However, a large proportion of the published information regarding histopathological effects of contaminants on aquatic organisms is nonquantal observations that depend heavily on who interprets the data (Dillon and Gibson 1986). It is recommended that histopathology testing be conducted only for specific toxicity testing for special problem cases.

Aryl hydrocarbon hydroxylase (AHH) induction bioassay. The AHH assay may have potential as a screening bioassessment tool. The assay detects the presence of toxic substances capable of inducing the hepatic mixed function oxidase enzyme system in rat hepatoma cell lines. Using tissue extracts of fish, Casterline et al. (1983) showed that AHH activity was positively correlated with tissue levels of polyhalogenated contaminants. The reaction is calibrated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and reports are given in TCDD equivalents. The halogenated dioxins, dibenzofurans, and biphenyls have been shown to induce AHH activity. The consensus of the phase 1 participants was that the screening tool needs extensive testing before use with sediments (Dillon and Gibson 1986).

Sister chromatid exchange (SCE). SCE has been described as an unscheduled exchange of similar genetic material between two sister chromatids of a single chromosome during cell division (Dillon and Gibson 1986). The assay is sensitive to several known mutagens and carcinogens, but it requires highly trained personnel and sophisticated equipment. Also, it is difficult to interpret the importance of the results unless supportive corroborative information on biological effects is also given (Dillon and Gibson 1986). Following testing of the technique using marine polychaetes and contaminated sediments, it was concluded that the test needed further research before SCE would be used for routine testing of sediments (Pesch et al. 1985).

Adenylate energy charge (AEC). The AEC refers to the relative concentration of adenylate nucleotides and is a measure of intracellular energy levels. The numerical quantitation of the end point is attractive from a regulatory perspective (Dillon and Gibson 1986). The phase I participants had reservations about the application of the assay because of the published

discrepancies of physiological health of organisms and AEC levels. At this time, this type of assay seems to need more research before it would be useful for sediment bioassays. Recent work with a marine bivalve and a marine polychaete exposed to highly contaminated sediments is a step forward (Zaroogian et al. 1985).

Microcosms. Microcosms address community changes over time, and they allow the degradation of the total community to be monitored. They probably should only be used for special problem projects because of their cost. Microcosms have regulatory application, but they are extremely time-consuming and expensive to conduct (Dillon and Gibson 1986). A large data set can be generated on the toxicity of sediments to animals indigenous to the disposal area. Decisions to conduct this type of assay would require a case-by-case examination of circumstances.

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APPENDIX A: TEST PROTOCOLS FOR TIERS II AND III

PART I: TIER II

Predictive Calculation

Neutral organic chemicals are distributed mainly in the lipids of organisms (Mackay 1982),* and in the organic carbon fraction of sediment (Karickhoff 1981). Neutral organic chemicals were calculated to have a preference factor of 1.72 for organism lipid over sediment organic carbon. Therefore, the maximum chemical concentration that would be found in an organism's lipid at equilibrium would be 1.72 times the concentration of the chemical in the sediment organic carbon. This calculated maximum is referred to as the lipid bioaccumulation potential (LBP) (McFarland and Clarke 1987). The equation for LBP is as follows:

$$LBP = 1.72 \frac{C_s}{fOC} \qquad (A1)$$

where

LBP = equivalent concentration in organism lipid in the same units of concentration as C_s

C_s = concentration of chemical in the sediment (any units of concentration may be used)

fOC = decimal fraction organic carbon content of the sediment

LBP would indicate maximum bioaccumulation potential in the lipid of any organism. It is more appropriate to convert LBP to a whole-body bioaccumulation potential (WBP) . This is done by multiplying the LBP by the organism's lipid content (expressed as a decimal fraction of wet weight), as determined by lipid analysis or from reported data:

$$WBP = LBP\,(fL) \qquad (A2)$$

where

WBP = maximum whole-body bioaccumulation potential in the same units of concentration as LBP

fL = decimal fraction of an organism's lipid content

If the calculated WBP is acceptable, then the sediment evaluation can be terminated.

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* See References at the end of the main text.

Acute Test Protocol

Acute lethality test (*Daphnia/Chironomus*): Adapted from Ziegenfuss and Adams (1985).

Procedure. The procedure would determine acute toxic effects of chemicals sorbed to sediments using *Daphnia magna* and *Chironomus tentans* in a 48-hr period (Ziegenfuss and Adams 1985). Each treatment would be replicated four times using five *D. magna* and five *C. tentans* per replicate, for a total of 20 *D. magna* and 20 *C. tentans* per treatment.

D. magna (<24 hr old) and *C. tentans* larvae (second instar) would be exposed to a reference sediment, a control sediment, and five concentrations of the sediment to be tested (proportionally diluted (w/w) with reference sediment) under static conditions at 22° C. A 50-g sample of sediment and 200 ml of water would be placed in a wide-mouth polycarbonate centrifuge bottle, shaken for 24 hr, and centrifuged for 15 min at 2,000 rpm (500 × G). Mortality checks would be made at 24 and 48 hr for *D. magna* but only at 48 hr for *C. tentans*. The EC50 (median effective concentration) and the NOEC (no observed effect concentration; mortality would be the effect noted) would be determined (American Public Health Association (AHPA) 1985; US Environmental Protection Agency/US Army Corps of Engineers (USEPA/CE 1977); Litchfield and Wilcoxon 1949).

Tests would be started after five concentrations of suspected toxic sediment have been prepared (Appendix C). Suggested concentrations (geometric series) are 100, 50, 25, 12.5, and 6.25 percent of the test sediment. Dilutions would be made with reference sediment. One hundred percent reference sediment and a control sediment would also be tested. Tests would be conducted in 250-ml clear polycarbonate centrifuge bottles (Fisher Scientific #05-430-53, or equivalent). Four replicates would be run per each concentration with 50 g of sediment (dry weight equivalent) and 200 ml of dilution water. Bottles would be capped and mechanically shaken for 24 hr, then centrifuged for 15 min at 2,000 rpm (500 × G).

Following centrifugation, bottles would be uncapped, and water chemistry measurements of temperature, dissolved oxygen concentration, and pH would be taken.

Test organisms would be placed in test bottles below the surface film in a random order, with midges (*C. tentans*) added first, one midge at a time. Each midge would be given time to burrow in the sediment before the next midge is added. This procedure would reduce the incidence of the midges attacking each other. Midges used would be between 10 and 14 days of age (second instar). Five midges would be added to each bottle, giving a total of 20 per test concentration. After all the midges are added, five daphnids (<24 hr) would then be added to each bottle in a random order.

The bottles would be left uncapped and exposed to a photoperiod of 16 hr dark/8 hr light at an intensity of 200-400 lux (18-36 foot candles). Test organisms would be fed daily during the test. Care would be taken so excess food would not be added to the test bottles. Otherwise, organisms would be disturbed as little as possible. Daphnid mortality checks would be made at 24 hr by counting survivors in each bottle. Organisms capable of movement would be considered alive for both species. Moribund organisms would be considered dead.

Test termination. The final 48-hr mortality check of daphnids would be made while removing the animals from the test bottles by pipette.

Water quality measurements (temperature, dissolved oxygen concentration, pH, alkalinity, and hardness) would be taken after the daphnids were removed but before the midge larvae were removed.

Any water samples for chemical analysis would be taken before the larvae were removed.

The water remaining would be carefully decanted from the bottles, and discarded midge larvae would be picked from the sediment and counted for survival.

Sediment and interstitial water chemical analysis can be run following midge removal by centrifuging the wet sediment at 9,000 × G. The resulting supernatant and sediment pellet would then be available for analysis. Soil/water partitioning coefficients would be determined by dividing the chemical concentration of the sediment by the chemical concentration of the water. A comparison to the chemical concentration of the previously removed surface water is also possible.

Calculations. Sediment test concentrations and the corresponding percentage mortality data obtained from the test would be used to calculate the 48-hr EC50 and its 95-percent confidence interval. Calculation can be accomplished by hand or by a computer program designed to calculate the EC50 by means of probit analysis, moving averages, and binomial analysis (APHA 1985; Litchfield and Wilcoxon 1949; Stephan,* Appendix G). The method producing the smallest 95-percent confidence interval would be the method of analysis reported.

If the soil/water partition coefficient is desired for a specific chemical known to occur in the sediment, it would be calculated by:

$$K_p = \frac{\text{chemical concentration of soil}}{\text{chemical concentration of water}} \qquad \text{(A3)}$$

Chemical concentrations in soil and water must be in the same units, such as mg/kg or mg/L. The carbon-normalized partition coefficient is calculated by:

$$K_{oc} = \frac{K_p}{\text{\% organic carbon of soil expressed as a decimal}} \qquad \text{(A4)}$$

Quality assurance. Criteria for rejection of a test include the following:

a. More than 10 percent of the organisms in the reference sediment or control sediment die.

b. Temperature deviation exceeds 3° C from 22° C.

c. Dissolved oxygen drops below 40 percent of saturation.

d. pH deviates by more than one pH unit.

e. A 50-percent mortality level is exceeded at all test concentrations.

Reporting results. Reported results should include the following:

a. Name of test, investigator, laboratory, and the date of testing.

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* Personal Communication, 1982, C.E. Stephan, Environmental Research Laboratory, US Environmental Protection Agency, Duluth, MN.

b. A brief description of the test sediment and the reference sediment, including the source of the sediments, collection method, storage method, and length of storage.

c. The source of dilution water and its chemical characteristics plus a brief description of any pretreatment.

d. Brief descriptions of the sources of *D. magna* and *C. tentans* and their history.

e. A brief description of the experimental design and a summary of methods.

f. All methods and results for water quality tests.

g. All methods and results of statistical data analysis.

h. Any unusual observations regarding the test, or any deviations from described protocols, or any other relevant information.

i. Copies of all raw data.

Changes in Methodology. Any modifications to the described protocol will be described in a written statement along with the reason or reasons for the proposed change. Such changes will be submitted to the project director, and written approval will be obtained before proceeding. Documentation relating to the request for change(s) and the approval for said changes will be appended to the method and included in the final report.

Ames test

The Ames test requires facilities equipped for bacteriological testing and sterile technique. Mutagenic chemicals are used in the procedures as controls, and appropriate handling procedures to protect human health are required.

The use of the Ames test for mutagenic testing is described in detail in papers by Ames, Lee, and Durston (1973); Ames et al. (1973); Ames, McKann, and Yamasaki (1975); Maron and Ames (1983); and by the USEPA (1983a). Application of the Ames test to sediment testing is described by Allen, Noll, and Nelson (1983). Extraction procedures given by Butler et al. (1985) may prove useful and have been used in conjunction with the Ames test. The process is too complex to present in adequate detail in this report, and the published approach is straightforward. Careful records of bacterial strains, culture media, and results are required.

PART II: Tier III

Chronic Test Protocols

***Daphnia magna* life cycle test**
Adapted from Nebeker et al. (1984)

Procedures. The procedure would determine chronic toxic effects of chemicals sorbed to sediments using *Daphnia magna* in a 10-day period (Nebeker et al. 1984). A chronic toxicity test with this organism would not be started until a 48-hr EC50 had been determined with the animals fed during the test. Each treatment would be replicated twice with 20 *D. magna* per replicate, for a total of 40 animals per treatment.

D. magna (5 days old) would be exposed for 10 days in glass jars with 2.5 L of water plus 500 ml of suspected toxic test sediment at 22° C. The exposure under static conditions would consist of a reference sediment, a control sediment, and five concentrations of the sediment to be tested (proportionally diluted (w/w) with reference sediment). At the end of 10 days, surviving adults and young would be counted. The EC50 and the NOEC (mortality would be the effect noted) would be determined for surviving adults (APHA 1985; USEPA/CE 1977; Litchfield and Wilcoxon 1949). The number of young produced for each test concentration would be reported (Nebeker et al. 1984).

Tests would be started after five concentrations of suspected toxic sediment have been prepared (Appendix C). Suggested concentrations (geometric series) are 100, 50, 25, 12.5, and 6.25 percent of the test sediment if the undiluted test sediment did not induce mortalities of 50 percent or more in the acute exposure. Dilutions would be made with reference sediment. A 100-percent reference sediment sample and a control sediment would also be tested. Each test concentration would be run in duplicate. Tests would be conducted in 4-L glass or clear polycarbonate jars with 2.5 L of water and 500 ml of test sediment. Water and sediment would be added to jars and would be allowed to settle for 24 hr prior to the addition of *Daphnia*. The test would be started with the addition of twenty 5-day-old daphnids to each jar in a random order along with food at the rate of 2 mg/L solids. Food should be added every other day for the duration of the test. Aeration of the containers would be accomplished with a glass-tipped air line 4 cm below the surface. The air stream should be sufficient to aerate the water yet not disturb the test sediment.

The jars would be covered with screening but would otherwise be open to the atmosphere. A photoperiod of 16 hr dark/8 hr light at an intensity of 200-400 lux (18-36 foot candles) would be used. Test organisms would be fed during the test. Care would be taken so excess food would not be added to the test jars. Dissolved oxygen and temperature would be monitored daily. Otherwise, organisms would be disturbed as little as possible. Daphnid mortality checks would be made at 10 days by counting survivors in each jar. Organisms capable of movement would be considered alive. Moribund organisms would be considered dead.

Test termination. The check for survivors and the enumeration of adults and young would be made at the end of the 10-day period. Water and fine solids would be poured through a 0.5-mm mesh screen. The bulk of the sediment would be left in the jar. Surviving adults and young daphnids would be gently rinsed and transferred to a bowl of clean water for counting.

Water quality measurements (temperature, dissolved oxygen concentration, pH, alkalinity, and hardness) would be taken before the daphnids are removed, using great care not to aspirate daphnids into sample containers.

Any water samples for chemical analysis would be taken before the daphnids are removed.

If concentration determinations of selected chemicals in the interstitial water and in the sediment are desired, the remaining sediment would be mixed to a uniform consistency, and a sample would be placed in a centrifuge tube and centrifuged at 9,000 × G for 20 min. The supernatant and the pellet would then be analyzed for the selected chemical. Soil/water partitioning coefficients would be determined by dividing the sediment chemical concentration by the water chemical concentration. A comparison to the chemical concentration in the previously removed surface water is also possible.

Calculations. Test concentrations of test sediment and the corresponding mortality data obtained from adults surviving the test would be used to calculate the 10-day EC50 and its 95-percent confidence interval. Calculation can be accomplished by hand or by a computer program designed to calculate the EC50 by means of probit analysis, moving averages, and binomial analysis (APHA 1985; Litchfield and Wilcoxon 1949; Stephan 1982,* Appendix G). The method producing the smallest 95-percent confidence interval would be the method of analysis reported.

The number of young daphnids would be reported for each test. Statistical evaluations by two-way analysis of variance and Dunnett's T test would be used to evaluate the effects of a sediment on the survival and growth. The analysis of variance provides information on effects among replicates and on whether there are differences among the treatments. The Dunnett's T test is used to determine whether one specific treatment is significantly different from the reference sediment.

Quality assurance. Criteria for rejection of a test include the following:

a. More than 10 percent of the adults in the reference sediment or control sediment die.

b. Temperature deviation exceeds 3^{o} C from 22^{o} C.

c. Dissolved oxygen drops below 40 percent of saturation.

d. pH deviates by more than one pH unit.

e. Mortality exceeds 50 percent at all test concentrations.

Reporting results. Reported results should include the following:

a. Name of test, investigator, laboratory, and the date of testing.

b. A brief description of the test sediment and the reference sediment, including the source of the sediments, collection method, storage method, and length of storage.

c. The source of dilution water and its chemical characteristics, plus a brief description of any pretreatment.

d. A brief description of the source of *D. magna* and the history of the specimens.

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* Personal Communication, 1982, C.E. Stephan, Environmental Research Laboratory, USEPA, Duluth, MN.

e. A brief description of the experimental design and a summary of methods.

f. All methods and results for water quality tests.

g. All methods and results of statistical data analysis.

h. Any unusual observations regarding the test, or any deviations from described protocols, or any other relevant information.

i. Copies of all raw data.

Changes in methodology. Any modifications to the described protocol will be described in a written statement along with the reason or reasons for the proposed change. Such changes will be submitted to the project director, and written approval will be obtained before proceeding. Documentation relating to the request for change(s) and the approval for said changes will be appended to the method and included in the final report.

***Chironomus tentans* partial life cycle test**
Adapted from Mosher, Kimerle, and Adams (1982)

Procedure. The procedure would determine chronic toxic effects of chemicals sorbed to sediments using *Chironomus tentans* in a 14-day period. A chronic toxicity test with this organism would not be started until a 48-hr EC50 had been determined with the animals fed during the test. Each treatment would be replicated twice using 25 *C. tentans* per replicate, for a total of 50 animals per treatment.

C. tentans would be exposed over 14 days of their life cycle (second to fourth instar) in glass test chambers with 2 L of water plus 100 g (dry equivalent) of the suspected test sediment under static and flow-through conditions at 22° C. The exposure would consist of a reference sediment, a control sediment, and five concentrations of the sediment to be tested (proportionally diluted (w/w) with reference sediment). Larvae would be fed daily. At the end of 14 days, survivors would be counted and weighed. The EC50 and the NOEC would be determined for surviving larvae (APHA 1985; USEPA/CE 1977; Litchfield and Wilcoxon 1949). Effects on growth and survival would be determined for each treatment. Partitioning coefficients could be determined for each test in the static exposure.

Test chambers would be aquaria constructed of glass and silicone rubber with a volume of 3 L. Dimensions would be 20.5 by 12.5 by 14.5 cm with a 12.5-by-4.5 cm piece of fine mesh stainless steel screen positioned on the upper end of one side. This overflow screen prevents the escape of larvae and maintains the test solution at 2 L. In the flow-through system, constant-head flow splitters would deliver dilution water at a rate of 8 to 12 L per day.

Second instar larvae of *C. tentans* are available 12-16 days after fresh egg masses are placed in rearing pans (Appendix F). Eggs should hatch in 2 days, and 10-day-old larvae are presumed to be in the second instar. Most larvae will remain in the second instar through the 14th day of age. Larvae older than 14 days should be discarded.

Tests would be started after five concentrations of suspected toxic sediment have been prepared (Appendix C). Suggested concentrations (geometric series) are 100, 50, 25, 12.5, and 6.25 percent of the test sediment if the undiluted test sediment did not induce mortalities of 50 percent or more in the acute exposure. Dilutions would be made with reference sediment. A 100-percent reference sediment sample and a control sediment would also be tested. Each test

concentration would be run in duplicate. Tests would be conducted in 3-L glass aquaria with 2 L of water and 100 g dry equivalent of sediment.

Static tests can be conducted in an area that meets the general facilities requirements (Appendix D). Flow-through tests must have an adequate temperature-controlled water supply with constant-head flow splitters to ensure equal flow to all test chambers.

Test tanks would be set up 3 days before testing begins. This time would allow the static tanks to come to equilibrium and the flow-through tanks to flush out contaminants desorbed from the sediment during mixing. Any floating debris should be skimmed from the surface before the test begins.

The test would begin with the addition of midge larvae to the test chambers. It is recommended that flow-through and static tests be started on different days to assure sufficient time to complete all tasks. Care would be taken to introduce larvae below the surface tension of the water. Each midge would be given time to burrow in the sediment before the next midge is added. Test chambers should be inspected for larvae trapped in the surface tension several hours after the test is started. These larvae should be discarded and replaced with healthy larvae.

Dissolved oxygen concentrations would be measured on days 0, 1, 4, 7, 10, and 14. Flow-through aquaria should not need supplemental oxygenation. Depending on the dissolved oxygen measurements, static aquaria would be gently aerated 1 day after the larvae have been introduced, or sooner. This aeration would use a glass-tipped air line 4 cm below the surface. This aeration would not disturb the sediment. Dissolved oxygen should not fall below 50 percent saturation.

Temperature should be 22° C $\pm$ 3° C and would be measured daily.

Larvae would be fed once per day during the course of the test. Approximately 50 mg of dry solids (Appendix F) would be administered (in a 0.5-ml suspension of food and water) to each aquarium. Excess food will promote the growth of fungus and should not be allowed to accumulate on the sediment. If excess food accumulates, feeding should be reduced.

Water quality determinations for hardness, alkalinity, pH, and conductivity would be measured before larvae are added to the test chambers and on days 7 and 14.

Test termination. Any water samples for chemical analysis would be taken before the larvae are removed.

The water in the test chambers would be carefully decanted at the end of the 14-day period. Larvae would be picked from the sediments, cleaned of foreign matter, counted, and placed in a weight boat of known dry weight. Midge bodies would be dried at 100° C for 2 hr, then weighed to determine the net dry weight of the midges from each test chamber.

If concentration determinations of selected chemicals in the interstitial water and in the sediment are desired, the remaining sediment would be mixed to a uniform consistency, and a sample would be placed in a centrifuge tube and centrifuged at 9,000 $\times$ G for 20 min. The supernatant and the pellet would then be analyzed for the selected chemical. Soil/water partitioning coefficients would be determined by dividing the sediment chemical concentration by the water chemical concentration. A comparison to the chemical concentration in the previously removed surface water is also possible.

Midge bodies may be analyzed for selected chemicals, and bioconcentration factors may be determined. It would not be suitable to analyze for volatile organics on oven-dried midge bodies.

If volatile or labile compounds are involved, surviving midges will need to be subsampled for determining chemical concentrations and total dry weight of survivors.

Calculations. Test concentrations of test sediment and the corresponding percent mortality data obtained from the test would be used to calculate the 14-day EC50 and its 95-percent confidence interval. Calculation can be accomplished by hand or by a computer program designed to calculate the EC50 by means of probit analysis, moving averages, and binomial analysis (APHA 1985; Litchfield and Wilcoxon 1949; Stephan,* Appendix G). The method producing the smallest 95-percent confidence interval would be the method of analysis reported.

Statistical evaluations by two-way analysis of variance and Dunnett's T test would be used to evaluate the effects of a sediment on the survival and growth of *C. tentans*. The analysis of variance provides information on effects among replicates and on whether there are differences among the treatments. The Dunnett's T test is used to determine whether one specific treatment is significantly different from the reference sediment.

If the sediment/water partition coefficient is desired for a specific chemical known to occur in the sediment, it would be calculated by:

$$K_p = \frac{\text{chemical concentration of soil}}{\text{chemical concentration of water}} \qquad \text{(A3, bis)}$$

Chemical concentrations in sediment and water must be in the same units, such as mg/kg or mg/L. The carbon normalized partition coefficient is calculated by:

$$K_{oc} = \frac{K_p}{\text{\% organic carbon of soil expressed as a decimal}} \qquad \text{(A4, bis)}$$

Bioconcentration factors (BCF) are determined by:

$$BCF = \frac{\text{concentration in organism}}{\text{concentration in sediment}} \qquad \text{(A5)}$$

Quality assurance. Criteria for rejection of a test include the following:

a. More than 15 percent of the organisms in the reference sediment or control sediment die.

b. Temperature deviation exceeds 3^{o} C from 22^{o} C.

c. Dissolved oxygen drops below 50 percent of saturation.

d. pH deviates by more than one pH unit.

e. None of the test concentrations have a statistically significant effect on the biological factors measured (survival and growth). Alternatively, all of the test concentrations have a statistically significant effect on the biological factors measured (survival and growth).

f. Analytical measurements of test solutions must not be extremely variable.

* Personal Comunication, 1982, C.E. Stephan, Environmental Research Laboratory, US Environmental Protection Agency, Duluth, MN.

Reporting results. Reported results should include the following:

a. Name of test, investigator, laboratory, and the date of testing.

b. Brief descriptions of the test sediment and the reference sediment, including the source of the sediments, collection method, storage method, and length of storage.

c. The source of dilution water and its chemical characteristics, plus a brief description of any pretreatment.

d. A brief description of the source of *C. tentans* and their history.

e. A brief description of the experimental design and a summary of methods.

f. All methods and results for water quality tests.

g. All methods and results of statistical data analysis.

h. Any unusual observations regarding the test, or any deviations from described protocols, or any other relevant information.

i. Copies of all raw data.

Changes in methodology. Any modifications to the described protocol will be described in a written statement, along with the reason or reasons for the proposed change. Such changes will be submitted to the project director, and written approval will be obtained before proceeding. Documentation relating to the request for change(s) and the approval for said changes will be appended to the method and included in the final report.

***Hyalella azteca* partial life cycle test**
Adapted from Nebeker et al. (1984)

Procedures. The procedure would determine chronic toxic effects of chemicals sorbed to sediments using *Hyalella azteca* in a 28-day period (Nebeker et al. 1984). Each treatment would be replicated twice using 50 *H. azteca* per replicate, for a total of 100 animals per treatment. A chronic toxicity test with this organism would not be started until a 48-hr EC50 using *Chironomus tentans* has been determined.

Adult *H. azteca* would be exposed in 20-L glass aquaria with 2 to 3 cm of sediment on the bottom with an overlay of 15 cm of water at 22° C. The exposure under static conditions would consist of a reference sediment, a control sediment, and five concentrations of the sediment to be tested (proportionally diluted (w/w) with reference sediment). Animals would be fed twice a week during the 28-day period. At the end of 28 days, surviving adults and young would be screened from the sediment and counted.

The EC50 and the NOEC (mortality of adults and number of young produced would be the effects noted) would be determined for surviving adults (APHA 1985; USEPA/CE 1977; Litchfield and Wilcoxon 1949). Effects on reproduction and survival would be determined for each treatment. Partitioning coefficients could be determined for each test in the static exposure.

Animals for tests would be of the same age, from the same culture conditions (22° C), and from a single source. Adult *H. azteca* of 55 days of age would be used to start the test. These animals would be near the end of their first reproductive cycle. During the test, they would be expected to release their broods, molt, and mate. The juveniles would not reproduce during the testing period.

Tests would be started after five concentrations of suspected toxic sediment have been prepared (Appendix C). Suggested concentrations (geometric series) are 100, 50, 25, 12.5, and 6.25 percent of the test sediment if the undiluted test sediment did not induce mortalities of 50 percent or more in the acute exposure of *C. tentans*. Dilutions would be made with reference sediment. A 100-percent reference sediment and a control sediment would also be tested. Each test concentration would be run in duplicate. Tests would be conducted in 20-L glass aquaria with 2-3 cm of sediment on the bottom and an overlay of 15 cm of water.

Static tests can be conducted in an area that meets the general facilities requirements (Appendix D). Test tanks would be set up 3 days before testing begins.

The test would begin with the addition of 50 adult amphipods (55-days old) to each of the test chambers.

Dissolved oxygen concentrations would be measured on days 0 and 1, then every third day thereafter. Static aquaria would be gently aerated 1 day after the amphipods have been introduced with a glass-tipped air line 4 cm below the surface. This aeration would not disturb the sediment. Dissolved oxygen should not fall below 50-percent saturation. Water lost to evaporation would be replaced with distilled water.

Temperature should be 22^{o} C $\pm$ 3^{o} C and would be measured daily.

Amphipods would be fed twice per week during the course of the test. Approximately 50 mg dry food (Appendix F) would be administered in a 100-ml suspension of food and distilled water to each aquarium. Excess food will promote mortalities. If excess food accumulates, feeding should be reduced proportionally.

Water quality determinations for hardness, alkalinity, pH, and conductivity would be measured before amphipods are added to the test chambers and on days 7, 14, 21, and 28.

Test termination. Any water samples for chemical analysis would be taken before the amphipods are screened from the sediment.

The water in the test chambers would be carefully siphoned without disturbing the bottom sediment at the end of the 28-day period. Amphipods would be screened from the sediments, rinsed of foreign matter, and counted.

If concentration determinations of selected chemicals in the interstitial water and in the sediment are desired, the remaining sediment would be mixed to a uniform consistency, and a sample would be placed in a centrifuge tube and centrifuged at 9,000 $\times$ G for 20 min. The supernatant and the pellet would then be analyzed for the selected chemical. Soil/water partitioning coefficients would be determined by dividing the sediment chemical concentration by the water chemical concentration. A comparison to the chemical concentration in the previously siphoned surface water is also possible.

Adult amphipod bodies may be analyzed for selected chemicals, and bioconcentration factors may be determined. If the test requires bioconcentration analysis, after screening the animals from the sediment they would be placed in clean water overnight to allow gut clearance, then frozen for later analysis.

Calculations. Test concentrations of test sediment and the corresponding survival and reproduction data obtained from the test would be used to calculate the 28-day EC50 and its 95-percent confidence interval. Calculation can be accomplished by hand or by a computer program designed to calculate the EC50 by means of probit analysis, moving averages, and binomial

analysis (AHPA 1985; Litchfield and Wilcoxon 1949; Stephan 1982,* Appendix G). The method producing the smallest 95-percent confidence interval would be the method of analysis reported.

Statistical evaluations by two-way analysis of variance and Dunnett's T test would be used to evaluate the effects of a sediment on the survival and reproduction of *H. azteca*. The analysis of variance provides information on effects among replicates and on whether there are differences among the treatments. The Dunnett's T test is used to determine whether one specific treatment is significantly different from the reference sediment.

If the sediment/water partition coefficient is desired for a specific chemical known to occur in the sediment, it would be calculated by:

$$K_p = \frac{\text{chemical concentration of soil}}{\text{chemical concentration of water}} \qquad \text{(A3, bis)}$$

Chemical concentrations in sediment and water must be in the same units, such as mg/kg or mg/L. The carbon-normalized partition coefficient is calculated by:

$$K_{oc} = \frac{K_p}{\text{\% organic carbon of soil expressed as a decimal}} \qquad \text{(A4, bis)}$$

BCF are determined by:

$$BCF = \frac{\text{concentration in organism}}{\text{concentration in sediment}} \qquad \text{(A5, bis)}$$

Quality assurance. Criteria for rejection of a test:

a. More than 10 percent of the adults in the reference sediment or control sediment die.

b. Temperature deviation exceeds 3° C from 22° C.

c. Dissolved oxygen drops below 50 percent of saturation.

d. pH deviates by more than one pH unit from 7.0.

e. None of the test concentrations have a statistically significant effect on the biological factors measured (survival and reproduction), or all of the test concentrations have a statistically significant effect on the biological factors measured (survival and reproduction).

f. Analytical measurements of test solutions must not be extremely variable.

Reporting results. Reported results should include the following:

a. Name of test, investigator, laboratory, and the date of testing.

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* Personal Communication, 1982, C.E. Stephan, Environmental Research Laboratory, US Environmental Protection Agency, Duluth, MN.

b. A brief description of the test sediment and the reference sediment, including the source of the sediments, collection method, storage method, and length of storage.

c. The source of dilution water and its chemical characteristics plus a brief description of any pretreatment.

d. A brief description of the source of *H. azteca* and their history.

e. A brief description of the experimental design and a summary of methods.

f. All methods and results for water quality tests.

g. All methods and results of statistical data analysis.

h. Any unusual observations regarding the test, any deviations from described protocols, or any other relevant information.

i. Copies of all raw data.

Changes in methodology. Any modifications to the described protocol will be described in a written statement along with the reason or reasons for the proposed change. Such changes will be submitted to the project director, and written approval will be obtained before proceeding. Documentation relating to the request for change(s) and the approval for said changes will be appended to the method and included in the final report.

Laboratory Determination of Bioaccumulation Potential

Analysis of survivors from chronic exposures can provide indications of whether a contaminant will bioaccumulate in a dose-dependent fashion. However, this analysis will not provide kinetic uptake information. In the case of exposure to sediment, exposing animals for increasing lengths of time would eventually allow a determination of the steady-state concentration. If the length of time for exposure is less than that required to reach a steady-state tissue concentration, it is still desirable to estimate what that maximum tissue concentration might be.

Laboratory determination of bioaccumulation potential requires that animals be exposed to sublethal sediment concentrations in a temporal fashion, ending at 28 days. It is recommended that a choice be made between *C. tentans* and *H. azteca* as the exposure animal. The exposures should be run in duplicate using previously described chronic procedures with 25 *C. tentans* per tank or 50 *H. azteca* per tank, for a total of 50 *C. tentans* per time period or 100 *H. azteca* per time period. However, if this protocol cannot produce sufficient tissue to perform the analyses for all the contaminants of concern, fathead minnows *Pimephales promelas* should be used. The testing protocol for *Pimephales promelas* is contained at the end of this section. A control sediment exposure, a reference sediment exposure, and exposures of a minimum of three sublethal dilutions of the test sediment (diluted with reference sediment) should be run. An equal number of animals would be analyzed for background concentration following gut clearance at the time the experiment is started. Exposures would run for 1, 2, 3, 5, 9, 15, 22, and 28 days.

Animals surviving the exposures would be enumerated and frozen for later chemical analysis. Sediments from the tanks would be centrifuged, and sediment/water partition coefficients would be determined as described under tier III, chronic procedures.

Calculations of potential maximum bioaccumulation can be accomplished by taking samples sequentially over a short time period of constant exposure, and using a simple kinetic model to project tissue concentrations at steady state such as shown below:

$$C_T = \frac{k_1 C_W}{k_2} \left(1 - e^{-k_2 t}\right) \quad \text{(A6)}$$

where

C_T = concentration of chemical in organism

k_1 = uptake rate constant

C_W = concentration of chemical in exposure medium

k_2 = elimination rate constant

t = time

Iterative nonlinear regression programs, such as those in the SAS NLIN procedure can be used to fit the data to the model (SAS Institute, Inc. 1982). As the exposure increases, the term $e^{-k_2 t}$ approaches zero, and

$$C_T = \frac{k_1 C_W}{k_2} = C_{ss} \quad \text{(A7)}$$

in which C_{ss} is the whole-body concentration of chemical at steady state. If steady state is not achieved during the laboratory exposure, C_{ss} can be projected by using the time-sequenced exposure data in a nonlinear regression method, as described. The projected achievable C_{ss} in an organism can then be compared with the maximum potential bioaccumulation WBP estimated from sediment chemistry and expressed as the proportion p of WBP projected at steady state:

$$p = \frac{C_{ss}}{WBP} \quad \text{(A8)}$$

If p is equal to 1, then all of the chemical of concern is bioavailable to the organism, but if p is less than 1, then the bioavailability is not absolute. Animals surviving the exposures would be enumerated and frozen for later chemical analysis. Sediments from the tanks would be centrifuged, and sediment/water partition coefficients would be determined as described under tier III, chronic procedures.

Procedures. Laboratory determination of bioaccumulation potential requires that animals be exposed to a sublethal sediment concentration. The exposure animal would be adult *P. promelas* (2-3 g each), cultured from stock within 7 days of each other. The exposures would be run in triplicate tanks, 30 *P. promelas* per tank, for a total of 90 *P. promelas* per sediment. A control sediment exposure, a reference sediment exposure, and exposures of the test sediment should be run. An equal number of animals (10 individuals per replicate times three replicates) would be

frozen for background concentration following gut clearance at the time the experiment was started. Exposures to the sediments would run for 10 and 30 days. Daily mortality checks would be made with dead or moribund individuals enumerated, removed, and discarded. At the end of 10 days, one sample, 10 surviving organisms, would be removed per tank and placed in clean water overnight to allow gut clearance, then frozen. At the end of 30 days, all surviving organisms would be removed per tank and placed in clean water overnight to allow gut clearance, then frozen.

The bioaccumulation exposures would be conducted in a flow-through system consisting of 39-L glass tanks. Each tank would receive 100 ml per min of 20° C water (see Appendix D for specifications of water). Prior to the start of the test, approximately 11 kg (5 cm depth) of sediment would be added to each test tank. The appropriate amount of water, approximately 15 cm depth, would be carefully added, the mixture would be allowed to settle for 24 hr, and then the flow-through system would be started and run for 24 hr before the test organisms are added.

The *P. promelas* would be fed daily during the exposures with fish flake food, in the amount equal to approximately 6 percent of the fish biomass in the tank. Feeding would be reduced if uneaten flake food remained on the bottom of the tank at the next feeding period.

Dissolved oxygen concentrations would be measured on days 0 and 1, then every third day thereafter.

Temperature would be 20° C $\pm$ 3° C and would be measured daily. Flow rate would also be measured daily and calibrated as necessary.

Water quality determinations for hardness, alkalinity, pH, conductivity, and suspended solids would be measured before the *P. promelas* are added to the test chambers and on days 5, 10, 20, and 30.

Any water samples for chemical analysis would be taken before the *P. promelas* are removed from the tanks.

Quality assurance. Criteria for rejection of a test include:

a. More than 10 percent of the adults in the reference sediment or control sediment die.

b. Temperature deviation exceeds 3° C from 20° C.

c. Dissolved oxygen drops below 50 percent of saturation.

d. pH deviates by more than one pH unit from 7.0.

e. Analytical measurements of test solutions must not be extremely variable.

APPENDIX B: USE OF REFERENCE TOXICANT

One of the major agreements that came from the phase 1 workshop was that any regulatory testing program would benefit by the routine use of standard reference toxicant bioassays to assess the sensitivity of test organisms. This type of testing not only reflects on the health and resiliency of laboratory animal stocks, it also reflects on the methods and procedures of the laboratory conducting the tests and facilitates interlaboratory comparisons.

In a review by Lee (1980), a number of chemicals were proposed for use as reference toxicants: sodium pentachlorophenate, hexavalent chromium, dodecyl sodium sulfate, sodium chloride, and phenol. Other compounds considered here include cadmium chloride, copper sulfate, and 1-octanol. For the purposes of this report, 1-octanol is the compound of choice. The rationale for this decision is given below.

Sodium pentachlorophenate was the chemical chosen by Lee (1980) as the most promising for reference toxicant testing. Also, it is currently being supplied by the US Environmental Protection Agency (USEPA) in small quantities for this type of testing. Its major attributes are that it gives consistent results, there is little danger of the test animals having preexposure experience from the environment, and it is easy to handle and detect. The major problem with the use of sodium pentachlorophenate is its human toxicity. For this last reason, it is not recommended.

The use of metals presents some problems with the affinities of cations for anionic species in the water and on test container walls. Hexavalent chromium appears to readily sorb to container walls (Lee 1980). Divalent cadmium and copper are easily complexed with carbonate compounds in the water and rapidly lost from solution; variations in water hardness between laboratories would make interlaboratory comparisons difficult. Although the use of prepared test water would facilitate interlaboratory comparisons, it would not solve complexation problems between cations and anions. Therefore, the use of metals as reference toxicants is not recommended.

The compounds dodecyl sodium sulfate and phenol are highly biodegradable. This biodegradability results in difficulties in maintaining test concentrations. Under static conditions, both are likely to be gone from test containers in 24 hr or less. Dodecyl sodium sulfate also varies in purity between manufacturers, and it is difficult to obtain consistent quality between batches. The use of these two compounds as reference toxicants is not recommended.

Sodium chloride presents test animals with an osmotic challenge. Its primary disadvantage is that its solubility limits preparation of stock solutions of sufficient concentration to be easily diluted in the range of toxic concentrations. Since the challenge is to the test organism and its osmoregulatory ability, the opportunity exists for adaptation to concentrations insufficient to induce rapid mortality. For these reasons, sodium chloride is not recommended as a reference toxicant.

The recommended reference toxicant for phase 3 testing is 1-octanol. This compound is biodegradable, it gives good reproducibility, and it is easily analyzed using flame ionization on a gas chromatograph (Knuth and Hoglund 1984). The mode of action is a nonspecific narcosis, and there is little chance of adaptation by the organisms. There is little chance of incidental or direct binding to other compounds or molecules in the environment of the test tank. Human toxicity is low. The pure liquid is irritating to skin and eyes, but the vapors are nonirritating to

the eyes and throat. The disadvantages are that it has limited water solubility (approximately 540 mg/L), that it is volatile (requiring a flow-through exposure), and that it is odorous.

Steady-state delivery of 1-octanol can be accomplished by floating approximately 0.25 in (0.6 cm) of 1-octanol in a 2,800-ml culture flask (Corning 4420 or equivalent) approximately three-fourths full of water. A siphon tube will deliver replacement water to the bottom of the flask. A magnetic stirring bar mixes the solutions and maintains a steady-state concentration of 1-octanol in the water at near 300 mg/L. Water with dissolved 1-octanol can be pumped from the flask at a rate of 10 ml/min (Broderius*).

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* Personal Communication, S.J. Broderius, Environmental Research Laboratory, USEPA, Duluth, MN.

APPENDIX C: MATERIAL HANDLING

Introduction

Sampling protocol and sample handling are important aspects of any environmental study to ensure that reliable, reproducible, and meaningful data are generated that will adequately address the purpose of the study. Plumb (1981) provides a very detailed and thorough discussion of sampling protocol and sample handling in connection with sampling of dredged material.

Sampling Protocol

The sampling design, by necessity, should be site-specific because major point sources, land-use activity, hydrological conditions, and sample variability fluctuate from area to area. Samples should be collected randomly within the area to be dredged and composited for the tests. In order to allow for a rerun of the tests or for proceeding to tier-IV testing, approximately 2-1/2 times the quantity necessary to perform one run of all the tests in tiers II and III should be collected. The number of samples required will depend on the required quantity and the number of samples that are necessary to adequately characterize the area. The number of samples necessary to characterize the area will depend on the degree of heterogeneity of the dredged material and will have to be determined on a case-by-case basis. A stratified sampling design may be more appropriate if the dredged material area is fairly large, if there are fairly clear-cut areas with different sediment quality, and if disposal decisions may vary with the different areas based on the outcome of the sediment testing. A variety of sample collection gears is available, each with its own limitations (Plumb 1981). Three gears are recommended for use based on site conditions:

Gear	Special Conditions	Recommended Use
Ponar (9 by 9)	Painted with metal-free marine paint and fitted with fine-meshed screen.	For use in most situations because of its ease of use.
Shipek	Painted with metal-free marine paint.	For limited use where current velocity or substrate conditions preclude the use of a ponar grab.
Corers (wide mouth)	Stainless steel.	For use where vertical heterogeneity is anticipated.

Sample Handling

Sample containers, preservation, and handling

Field. For shipping and storage, samples should be placed in either stainless steel containers, such as milk containers, or in linear polyethylene containers. The containers should be filled to

the top, leaving no air space. Excess water should be decanted to the extent possible without affecting the integrity of the sample, including the amount of interstitial water. Teflon or stainless steel spoons can be used to assist in transferring the material from the sampling device to the shipping and storage containers. However, handling in the field should be minimized to the extent possible. All sampling containers and equipment should be cleaned with the appropriate organic solvent and acid. The samples should be maintained on ice and delivered to the laboratory for processing within 24 hr of collection.

Laboratory. Samples should be logged in and processed immediately upon receipt within the laboratory. Samples should be homogenized in a Hobart or similar commercial mixer equipped with stainless steel paddle and bowl. This homogenization should occur in an inert atmosphere, such as argon, to prevent oxidation of the sediments. Portions of the homogenate should be taken for each of the types of testing required in tiers II and III. Portions taken for bulk chemical analysis of metals should be stored in nitric-acid-rinsed polyethylene bottles. Portions taken for organic analysis should be stored in glass bottles, with either a Teflon-lined or aluminum-foil-lined cap. The portions remaining for bulk chemical analysis and bioassessment testing should be stored in linear polyethylene bottles. Air spaces should not be left in the bottles. All portions extracted from the homogenate and the remaining homogenate should be stored at 4^{o} C until the tests are performed.

Quality Control

Normal chain of custody and other quality control procedures should be followed both in the field and laboratory (American Society for Testing and Materials 1985). Waterproof labels, marked with a water-indelible pen or pencil, should be used to label the samples.

Reference and Control Sediments

Control sediments should be contaminant-free, consisting of silica sand. Sediments suitable for use as a control are available commercially. The most important consideration in the control exposure is to maintain greater than 90 percent survival of the test organisms. Therefore, it may be necessary to consider the use of alternative measures (i.e., the sediments used to culture the organisms) to ensure that there will be greater than 90 percent survival in the controls.

Reference sediments are sediments that are relatively contaminant-free, with low toxicity and low contaminant bioavailability. The responses of test species to a reference sediment in a controlled experiment are considered the best possible laboratory simulation of biological responses to a sediment known to possess generally acceptable characteristics.

In determining a collection location and the suitability of a reference sediment, the following criteria should be used:

a. Reference sediments should be of known quality and/or should be analyzed for bulk chemistry to ensure that they are relatively contaminant-free.

b. Reference sediments should support a good, diverse population of benthic organisms, preferably including the test organisms used in the bioassay or their congeners.

c. Reference sediments should be similar in particle size to the test sediments.

d. If possible, reference sediments should be collected from within the same general system as the test sediments. If a proposed disposal site is known and has sediments that meet all of the criteria for use as a reference sediment, it should be selected as the source for the reference sediments.

Reference sediments should be collected and handled following the same protocol as described for the test sediments. The reference sediments should be collected at approximately the same time as the test sediments.

Dilution of the test sediments with reference sediments should be done on a dry weight equivalent basis. The mixtures should be thoroughly mixed prior to the addition to the test aquaria.

APPENDIX D: TEST FACILITY

The test facility should provide an area for testing that is isolated from normal traffic. This area should have its own separate controls for lighting, ventilation, and temperature. The ventilation system should be separate from the rest of the building or be constructed so that airborne contaminants such as smoke, dust, and solvent vapors do not enter the test area. Temperature control should provide a constant temperature with fluctuations being within 1° C. Lighting will be adjustable for automatic control of photoperiod, and a 15-30 min artificial dawn and dusk are desirable.

The water supply should provide uncontaminated water of constant quality. A minimal criterion for acceptable dilution water is that healthy, unfed *Daphnia* (<24 hr old) will survive for 48 hr in a static situation without signs of stress. Dilution water from wells or surface waters should not exceed the following specifications (Ziegenfuss and Adams 1985):

Particulate matter	20 mg/L
Total organic carbon or chemical oxygen demand	5 mg/L
Un-ionized ammonia	20 µg/L
Residual chlorine	3 µg/L
Total organophosphorus pesticides	50 ng/L
Total organophosphorus pesticides plus poly-chlorinated *biphenyls* (PCBs) or organic chlorine	50 ng/L
PCBs or organic chlorine	25 ng/L
Hardness (mg/L $CaCO_3$)	100 mg/L
pH	7.0-8.2
Boron, fluoride	100 µg/L each
Aluminum, arsenic, chromium, cobalt, copper, iron, lead, nickel, zinc	1 µg/L each
Cadmium, mercury, silver	100 ng/L each

Great Lakes waters that are not contaminated are acceptable. A natural surface water is considered to be of constant quality if monthly ranges of the hardness, alkalinity, and specific conductance are less than 10 percent of their respective averages and if the monthly pH range is less than 0.4 unit. Dechlorinated city water will be used only as a last resort. Municipal water supplies often contain unacceptably high concentrations of copper, lead, zinc, fluoride, chloride, or chloramines (Ziegenfuss and Adams 1985). Reconstituted water may be prepared by adding specified chemicals to highly purified distilled water. Instructions for the preparation of reconstituted water are given in American Society for Testing and Materials (ASTM) Standard Practice E 729-83 (ASTM 1985). Water delivery systems will be constructed of PVC pipe.

Construction materials and commercial equipment may contact stock, test, and dilution water used in the bioassays. These materials should not contain substances that can be leached or dis-

solved in aqueous solutions. Materials should be chosen so that the sorption of toxicants from sediments and water would be minimized. Glass, #316 stainless steel, high-density polyethylene, and perfluorocarbon plastics should be used to minimize leaching, dissolution, and sorption of toxicants. Compressed air will be supplied by an oil-free air compressor or filtered to the point that it is oil-free. Facilities for the refrigeration of sediment materials and food stocks will be provided. Freezers without automatic defrost cycles should be used for preserving test organisms for chemical analysis.

APPENDIX E: DAILY PROCEDURES

The daily procedures to be followed by laboratory personnel will fall into two areas; (a) maintaining animal stocks and cultures, and (b) conducting sediment toxicity tests. The first will involve routine maintenance of organisms for testing and the second will involve the conduct of tests. The two areas are not mutually exclusive, and they lead to overall quality control and quality assurance for toxicity testing. Accurate and conscientious record keeping is imperative.

Guidance for the standardized methods and good standards of laboratory practice are given in Standard Methods (American Public Health Association 1985), Biesinger et al. (in US Environmental Protection Agency 1986a, Appendixes F and H), and in 40 CFR (Code of Federal Regulations) Part 792 - Good Laboratory Practice Standards, Revised as of July 1, 1985, pp. 255-268. The major areas of concern are given in Table E1.

Routine cleaning of exposure chambers, pipettes/glass tubing, general glassware, food containers, culture chambers, test probes, and other specialized equipment should follow the following sequence: (a) phosphate-free detergent; (b) acetone or appropriate solvent rinse; (c) acid rinse (5-10 percent HNO_3); (d) distilled water rinse; and (e) dilution water rinse.

Table E1
Major Areas of Concern
for Daily Procedures

Animal Cultures
Water supply and routine water chemistries
Aquaria or holding tanks
Temperature control and photoperiod
Removal of excess food
Checking and recording observations of mortalities, disease, and prophylactic treatments
Feeding as appropriate
Maintenance of food stocks
Observations and records of normal and abnormal behavior
Control animal density in stock tanks
Maintain records of stocks and propagation
Production of known age animals for testing
Normal cleaning and sanitation
Conducting Toxicity Tests
Water supply and routine water chemistries
Aquaria or test chambers
Temperature control and photoperiod
Removal of excess food
Check for and record observations of mortalities and disease
Feeding as appropriate
Maintenance of food stocks
Observations and records of normal and abnormal behavior

APPENDIX F: ANIMAL CULTURE

General

A primary requisite for conducting sediment toxicity tests is an adequate source of healthy experimental organisms (American Public Health Association (APHA) 1985). Aquatic organisms are easily damaged or stressed during transport and may easily become stressed or diseased because of improper holding and culture conditions. In extreme cases, they may be diseased before shipping from a supplier. The use of any unhealthy organisms will produce unreliable test results. To reduce the incidence of problems, the best general guideline is to meet the needs of the organisms (Dillon and Gibson 1986); in many cases this can only be accomplished by culture on site. In any case, adequate nutrition and water quality are fundamental to the effort of producing disease-free and parasite-free animals with normal behavior patterns (Lawrence 1981).

A recommended procedure for all incoming animals and live food is to quarantine them for disease and parasites. It is also recommended that test organisms be checked for content of pesticides, heavy metals, and other toxic materials as appropriate (APHA 1985). Laboratory culture of invertebrates can be used to produce numerous organisms for testing. The animals then have a known history, life phase, and physiological condition that increase their worth in toxicity testing (Lawrence 1985).

Organisms must be kept in a stable reproducible environment with daily temperature fluctuations less than 1^{o} C. Dissolved oxygen should not fall below 60 percent (maintained near 100 percent for *Pimephales promelas*) of saturation, and nitrogenous waste products should not accumulate. Flow-through water volumes may need to approach 3 L per day per gram of organisms (APHA 1985). Gas supersaturation of tank water should not occur. This is likely to happen when cold water is warmed before addition to holding tanks.

Discussions of disease and parasite control, culture media, and specialized equipment are given in Standard Methods (APHA 1985), Lawrence (1981), and Biesinger et al. (in US Environmental Protection Agency (USEPA) 1986a).

Daphnia magna

Culture of *Daphnia magna* should be accomplished under static conditions following the procedures and recommendations given in papers by Leonhard and Lawrence (Lawrence 1981), Goulden et al. (1982), and Biesinger et al. (USEPA 1986a). Specifically, culture must be maintained at 22^{o} C $\pm$ 1^{o} C in a constant temperature bath, or room, with a 16-hr light/8-hr dark photoperiod and normal daylight intensity.

Feed must be trout food obtained from the USEPA Environmental Research Laboratory, Duluth, MN, plus cells of the algae *Selanastrum capricornutum* (USEPA 1986a). *S. capricornutum* is available from the American Type Culture Collection*, or the Starr Collection**.

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* Located at 12301 Parklawn Drive, Rockville, MD 20852 (ATC #22662).

** Located at Department of Biology, University of Texas at Austin, Austin, TX 78712 (U TEX 1648).

Careful records should be maintained so that sufficient animals of known age are available for testing. All surviving animals used in testing should be destroyed. Under no circumstances can they be reintroduced into culture.

Chironomus tentans

Chironomus tentans culture should follow the procedures and recommendations given in Townsend et al. (Lawrence 1981), Nebeker et al. (1984), Batac-Catalan and White (1982), and Monsanto's standard operating procedure EAS-82-50P-44 (Batac-Catalan and White 1982; Dillon and Gibson 1986). Specifically (Lawrence 1981; Batac-Catalan and White 1982; Dillon and Gibson 1986), the culture must be maintained at 22° C $\pm$ 1° C in a constant-temperature room or incubator with a wide spectrum light intensity of approximately 100 foot-candles in a 16-hr light/8-hr dark cycle. Food must be made from Tetra® conditioning food vegetable diet for tropical fish available from most aquarium suppliers.

Careful records should be maintained so that sufficient animals of known age are available for testing. All surviving animals used in testing should be either frozen and saved for analysis or destroyed. Under **no circumstances** can they be reintroduced into culture.

Hyalella azteca

Hyalella azteca culture should follow the procedures and recommendations given in papers by de March (Lawrence 1981) and Nebeker et al. (1984). Specifically, the culture must be maintained at 22° C $\pm$ 1° C in a constant-temperature room or incubator with a wide-spectrum light intensity of at least 55 uE/m/sec surface intensity in a 16-hr light/8-hr dark cycle. The light intensity is critical because the animals grow differently at different light intensities. As a guide, the maximum light intensity at water surface in natural waters in Manitoba, Canada, is 2,200 uE/m/sec (de March, in Lawrence 1981). Food must be Tetra-Min®B, available from most aquarium suppliers.

Careful records should be maintained so that sufficient animals of known age are available for testing. All surviving animals used in testing should be either frozen and saved for analysis or destroyed. Under **no circumstances** can they be reintroduced into the culture.

Pimephales promelas

Pimephales promelas culture should follow the procedures and recommendations given by Klemm, USEPA (1985a).

APPENDIX G: DATA EVALUATION

Responses of test animals to toxicants or toxic sediments can be classified as two types, quantal and quantitative. A quantal response is an either/or response. For example, in using death as an end point, an animal either dies during the test exposure or it does not. If animals respond to a variable degree, such as the amount of growth during a chronic exposure, the response is quantitative or graded (American Public Health Association (APHA) 1985).

By design, quantal tests estimate the concentration of toxic material affecting 50 percent of the test animals. The chosen end point can be death or some sublethal effect such as immobilization, hypersensitivity to disturbance, or an other abnormal behavior pattern. One could also choose a significant effect on factors such as growth and reproduction, physiological effects such as inhibitions of enzyme systems, and/or morphological effects on tissue structure (APHA 1985). The distinction between quantal and quantitative effects becomes somewhat fuzzy when using sublethal effects as end points. However, the main distinction is the choosing of a defined end-point in a range of responses rather than using the entire range. For responses other than death, it is recommended that median effective concentration (EC50) be used in place of LC50 and that the word or phrase describing the sublethal response be used in text descriptions of the results (APHA 1985). The term LC50 should be used with care since toxic sediments are generally composed of mixtures of compounds that may provide both synergistic and additive effects as well as modifiers that may prevent toxicity when a compound is present in "toxic concentrations." It is desirable to report 95-percent confidence limits for LC50, EC50, and no observed effect concentration (NOEC) estimates.

There are a number of issues associated with the analysis of toxicity test data and the interpretation of the results of that analysis. Many problems can be avoided by consulting with a qualified statistician before the experimental design phase and testing begin. The following points need to be considered prior to data analysis:

a. Statistics is not an end in itself; rather, it is a means of interpreting data and test results.

b. Investigators should always be attentive for statistically significant results that are biologically meaningless.

c. A diversity of opinion exists among scientists, among statisticians, and among scientists and statisticians about the acceptable methods for calculating estimates such as the EC50*.

d. The selection of an analytical method for analysis often depends upon the data obtained. This runs contrary to most statistical advice that studies be analyzed using "a priori" comparisons. Work by Stephan* suggests that in many cases certain approaches yield more valid results than others. This finding does not reduce the need for consulting with a statistician before testing begins.

e. The application of computer programs to facilitate analysis sometimes results in a blind acceptance of results. The use of analytical methods for analysis should be tempered with an understanding of the limitations of the methods employed.It is beyond the scope of this report to recommend specific statistical procedures for every possible contingency.

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* Personal Communication, 1982, C.E. Stephan, Environmental Research Laboratory, US Environmental Protection Agency (USEPA), Duluth, MN.

This report suggests that use of the given references and consultations with a statistician familiar with bioassays will provide adequate guidance (APHA 1985; USEPA/US Army Corps of Engineers 1977; Litchfield and Wilcoxon 1949; USEPA 1985a and b). When reporting results, the reasons for choosing and employing particular methods should be stated.

APPENDIX H: HEALTH AND SAFETY

General Precautions

Collection and analysis of sediment samples may involve significant risks to personal safety and health. The following discussion of safety requirements has been adapted from two sources: USEPA (1985a), and Standard Methods (American Society for Testing and Materials (ASTM) 1985). Personnel involved in the collection of the samples and the laboratory analysis should protect themselves from injury by taking all safety precautions necessary for the prevention of bodily injury and inhalation or absorption through skin contact with any corrosive or toxic substances. Prior to sample collection and laboratory work, personnel should make sure that all necessary safety equipment and materials have been obtained and are in good condition.

Field Precautions

All motor vehicles, trailers, and boats should comply with all State safety requirements and US Coast Guard regulations. All motorboats and vehicles should be equipped with fire extinguishers and first aid kits.

Field people should be trained in cardiopulmonary resuscitation and first aid.

Because the chemical and biological compositions of the sediments are usually only poorly known, the sediments should be considered potential health hazards, and exposure to them in both the field and the laboratory should be minimized.

Laboratory Precautions

The laboratory should have a safety program, including a training program for its personnel. Information on toxicity to humans and recommended handling procedures for the chemicals (ASTM 1985) that will be used during the testing should be studied before the tests are initiated.

The laboratory should have the following safety features (ASTM 1985a): (a) fire extinguishers, both water-type and dry chemical type, should be in readily accessible places; (b) fire blankets should also be readily accessible; (c) eye washes should be located near sinks; (d) safety glass and movable doors should be on conventional chemical hoods; (e) safety containers should be used to transport and store hazardous chemicals and wastes; (f) hazardous chemicals and wastes should be stored in a separate area away from the work area; (g) chemical spill kits should be available in all work and storage areas; (h) personal protective equipment and materials, including laboratory garments, gloves, safety shoes, and safety glasses, should be available and used by laboratory personnel; and (i) an adequate disposal plan should be prepared for the disposal of chemical wastes generated during the testing.

Adequate ventilation should be maintained throughout the laboratory, to minimize levels of airborne toxins. Periodic monitoring of air quality within the laboratory should be part of the laboratory's safety program. All activities that could result in impacts on air quality, including acid digestions, organic extractions, and cleaning of laboratory equipment and glassware with organic solvents, should be performed in an appropriate chemical hood.

Good personal hygienic and housekeeping practices are important safety factors and should be practiced by the personnel involved in the testing.

APPENDIX I: SUGGESTED PARAMETERS AND METHODS FOR BULK CHEMICAL ANALYSIS

Table I1
Parameter list, analysis methods,
and desired sensitivity for parameters

Parameter	Method	Citation	Detection Limit µg/g dry weight
Particle size	Sieve and pipette	Plumb 1981	1.00 %
Total solids	Gravimetric 160.3	USEPA 1983b	100.00
Volatile solids	Ashing method 160.4	USEPA 1983b	100.00
Total organic carbon	SW846 - EPA method 9060	USEPA 1986b	1.00
Percent moisture	Method 160.3	USEPA 1983b	0.10
Ammonia nitrogen*	Mod. elutriate with 350	USEPA 1983b	0.20 µg/L
Kjeldahl nitrogen	Kjeldahl digestion	Plumb 1981	10.00 µg/g
Total phosphates	Strong acid digestion	Plumb 1981	10.00 µg/g
Sulfides	Colorimetric	Plumb 1981	0.50 µg/g
Chemical oxygen demand	Digestion	Plumb 1981	10.00 µg/g
Oil and grease	Freon extraction	Plumb 1981	10.00 µg/g
Cyanide	SW846 - EPA method 9010	USEPA 1986b	0.50
Metals			
Arsenic	SW846 - EPA method 7060	USEPA 1986b	0.10
Cadmium	SW846 - EPA method 7131	USEPA 1986b	0.10
Chromium	SW846 - EPA method 6010	USEPA 1986b	1.00
Copper	SW846 - EPA method 6010	USEPA 1986b	1.00
Lead	SW846 - EPA method 7421	USEPA 1986b	1.00
Mercury	SW846 - EPA method 6010	USEPA 1986b	0.01
Nickel	SW846 - EPA method 6010	USEPA 1986b	1.00
Selenium	SW846 - EPA method 7740	USEPA 1986b	1.00
Zinc	SW846 - EPA method 6010	USEPA 1986b	1.00
Manganese	SW846 - EPA method 6010	USEPA 1986b	10.00
Chlorinated hydrocarbons**			µg/kg dry weight
Aldrin	Method 8080	USEPA 1986b	0.08
alpha BHC	Method 8080	USEPA 1986b	0.06

(Continued)

* Samples for ammonia analysis would be extracted following the modified elutriate procedure. A slurry of water and sediment (concentration of 150 g/L dry weight equivalent) would be prepared and mixed for 5 min. This mixture would then be centrifuged at 10,000 times gravity and the supernatant collected and analyzed immediately or within 24 hr, if acidified with H_2SO_4 to pH <2 and stored at 4° C, following US Environmental Protection Agency (USEPA) method 350.1 or equivalent.

** Samples will be extracted by USEPA method 3550. Instead of 30-g samples, 50 g will be used. The extract will be concentrated to 10 ml and cleaned by Gel Permeation Chromatography (GPC) using USEPA method 3640. The cleaned extract will be further concentrated to 1 ml and solvent exchanged to hexane. The hexane solution will be concentrated to 1 ml before analysis by USEPA method 8080. To alleviate problems associated with other contaminants, the extract will be further cleaned by Florosil column using USEPA method 3620.

Table I1 (Concluded)

Parameter	Method	Citation	Detection Limit µg/g dry weight
Chlorinated hydrocarbons (Continued)			
beta BHC	Method 8080	USEPA 1986b	0.12
delta BHC	Method 8080	USEPA 1986b	0.18
gamma BHC (Lindane)	Method 8080	USEPA 1986b	0.08
Chlordane	Method 8080	USEPA 1986b	0.28
DDD	Method 8080	USEPA 1986b	0.22
DDE	Method 8080	USEPA 1986b	0.08
DDT	Method 8080	USEPA 1986b	0.24
Dieldrin	Method 8080	USEPA 1986b	0.04
Endrin	Method 8080	USEPA 1986b	0.12
Endosulfan I	Method 8080	USEPA 1986b	0.28
Endosulfan II	Method 8080	USEPA 1986b	0.08
Heptachlor	Method 8080	USEPA 1986b	0.06
Heptachlor Epoxide	Method 8080	USEPA 1986b	1.70
Methoxychlor	Method 8080	USEPA 1986b	3.30
PCBs (aroclors 1016, 1221, 1232, 1242, 1248)	Method 8080	USEPA 1986b	1.20
PCBs (aroclors 1254 1260)	Method 8080	USEPA 1986b	2.40
Herbicides*			µg/g dry weight
2,4-D	Method 8150	USEPA 1986b	0.60
2,4,5-T	Method 8150	USEPA 1986b	0.10
2,4,5-TP (Silvex)	Method 8150	USEPA 1986b	0.08
Polynuclear Aromatic Hydrocarbons			
Anthanthrene	Meth. Extract/UV Analysis	Plumb 1981	0.10 µg/kg
Anthracene	Meth. Extract/UV Analysis	Plumb 1981	0.10 µg/kg
Benzanthracene	Meth. Extract/UV Analysis	Plumb 1981	0.10 µg/kg
Benoperylene	Meth. Extract/UV Analysis	Plumb 1981	0.10 µg/kg
Benzopyrenes	Meth. Extract/UV Analysis	Plumb 1981	0.10 µg/kg
Chrysene	Meth. Extract/UV Analysis	Plumb 1981	0.10 µg/kg
Coronene	Meth. Extract/UV Analysis	Plumb 1981	0.10 µg/kg
Fluoranthene	Meth. Extract/UV Analysis	Plumb 1981	0.10 µg/kg
Perylene	Meth. Extract/UV/Analysis	Plumb 1981	0.10 µg/kg
Phenanthrene	Meth. Extract/UV/Analysis	Plumb 1981	0.10 µg/kg
Pyrene	Meth. Extract/UV/Analysis	Plumb 1981	0.10 µg/kg
Phenolics	Distillation Chlorimetric	Plumb 1981	1.00 µg/kg

* Sample size for herbicide analysis will be increased to 100 g and the extract volume at the end of the preparation will be reduced to 1 ml.